

Natural history of liver fibrosis

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Liver fibrosis is the hallmark of all chronic liver diseases; failure to eliminate a deleterious agent (virus, toxic, autoantigen) leads to chronic inflammation with liver fibrosis and cirrhosis as an end point. Pathogenesis of cirrhosis is therefore close to other destructive fibrosis, such as chronic gastric ulcer, hypertrophic scar or pulmonary interstitial fibrosis.

The liver extracellular matrix

Liver fibrosis is the result of the accumulation of extracellular matrix components (ECM) with subsequent destruction of liver architecture and liver cell dysfunction. ECM is a complex network composed of three main groups of macromolecules: the collagens, the adhesive glycoproteins and the proteoglycans (1). Among the most abundant proteins are those of the collagen family (mainly type I, III, IV, V, VI and XVIII in the liver). From a histopathological point of view, these different collagen isotypes form either interstitial or fibrillar collagens (collagen type I, III and V) or basement membranes (type IV collagen). Among the glycoprotein family, most important are laminin (an other major constituent of basement membranes) and fibronectin. These molecules serve not only as a scaffold for epithelial liver cells but also have specific functions according to their modular architectures. Fibronectin is involved in cell attachment, differentiation and migration through its different domains, each involved in interaction with specific ECM component. Proteoglycans, the third major group of ECM molecules are composed of a protein backbone linked to sulfated polydisaccharides (glycosaminoglycans). They are located in the interstitium or in cell membrane, either transmembranous or pericellular. Liver ECM is also rich in cytokines, growth factors, or serum macromolecules that are sequestered into ECM through specific interactions with various components, mainly proteoglycans and adhesive glycoproteins. Transforming growth factor- β (TGF- β), a major growth factor involved in fibrogenesis is bound to ECM in an inactive form. Under certain circumstances, these molecules can be activated and serve as a local reservoir of active molecules.

Hepatic stellate cells (HSC) are the major cellular source of EOM molecules (2). They are located in the Disse's space. In the normal liver, the function of HSO is to store vitamin A. Under various stimuli (disruption of cell-cell or cell-matrix contacts, growth factors such as TGF- β , reactive oxygen species, lipid peroxidation products, acetaldehyde), these cell undergo phenotypical transformation to transitional cells with a myofibroblast phenotype. In this condition, HSC produce a large amount of the different EOM components. Endothelial cells but also fibroblasts of the portal tract are also involved in ECM production.

It is now feasible to isolate and grow HSC *in vitro*. This approach makes it possible to study in detail the multiple molecules involved in HSC activation as well as their signal transduction pathways. With this model, it has been showed that TGF- β is the major fibrogenic molecule inducing transcription of most of the EOM molecule-associated genes (collagen, fibronectin, laminin) and that platelet-derived growth factor (PDGF) is the major mitogenic molecule for HSC. Other cytokines and growth factors such as fibroblast growth factor-2 (FGF-2), interleukin-10 (IL-10) and interferon-gamma are

also involved in the regulation of these genes as well as in HSC proliferation. These studies have also allowed for the development and the study of antifibrogenic agents which have also been tested on different experimental models of liver fibrosis. Antioxidants, anti-sense or soluble receptors of growth factors are being tested, some of them with promising results.

How to evaluate liver fibrosis

Liver biopsy is the gold standard for assessing liver fibrosis. However, sample variability of fibrosis is a major drawback in fibrosis assessment. Liver fibrosis is a diffuse lesion, it is closely associated with liver regeneration so that areas of dense fibrosis may coexist with areas of liver cell regeneration leading to irregular distribution of fibrosis. Such variability in the distribution of fibrosis precludes the use of overly precise objective methods of fibrosis measurement on liver core biopsy (quantitative image analysis). The use of less accurate but more reproducible scoring systems, such as those proposed in the different scoring systems of chronic hepatitis, are much more adapted to assess fibrosis with confidence in clinical practice. Theoretically, the use of serum markers of liver fibrosis would allow a global assessment of the fibrogenesis process. Unfortunately there is no marker of fibrosis stage or fibrogenesis with sufficient sensitivity and specificity to have high positive or negative predictive values.

Can fibrosis regress

Strong biochemical arguments suggest that the liver contains enzymatic equipment allowing the destruction of ECM. Matrix metalloproteinases (MMP) are a family of proenzymes that can destroy the different ECM components (3). Several studies have shown overexpression of MMP in various conditions associated with liver fibrosis. MMP are produced by HSC, the same cell type involved in EOM production. These cells also produce tissue inhibitors of metalloproteinases (TIMP), a set of molecules that inhibit MMP activity. Therefore, evaluation of matrix degradation remains a difficult task.

There are, however, strong experimental and clinical arguments showing that liver fibrosis can regress. In the O014-induced liver cirrhosis in rat, the arrest of O014 administration allows a near complete fibrosis regression. At this point, it must be remembered that cirrhosis is composed of the association of both annular fibrosis and liver cell regeneration. It is also evident, from human and experimental studies, that liver regeneration begins very early after liver insult, well before the cirrhotic stage. There is now strong evidence showing that liver fibrosis regression can occur both from ECM degradation and liver regeneration. It can be hypothesized that at a stage of stable fibrosis (in the absence of ongoing fibrogenesis), liver cell regeneration can compress and disrupt fibrous septa leading to a restitution of near normal liver architecture with portal tract and centrilobular veins. As a matter of fact, a recent experimental study showed that gene therapy using a hyperglycemic-glycogenolytic factor (HGF) retroviral vector induced the complete disappearance of the cirrhotic architecture in an animal model of fibrosis.

Natural history of liver fibrosis Chronic hepatitis C as a paradigm

Chronic hepatitis C virus infection (HCV) is estimated to affect 170 million individuals worldwide. Thirty percent of these individuals will eventually develop cirrhosis. HCV can be lethal almost exclusively when it leads to cirrhosis. Therefore, an estimate of fibrosis pro-

gression represents an important surrogate end point for evaluation of the vulnerability of a given patient and for assessment of treatment impact on natural history. Activity grade is not very useful to predict fibrosis progression since there is no study demonstrating that activity is predictive of fibrosis progression independent of fibrosis stage and, in fact, fibrosis alone is the best marker of ongoing fibrogenesis (4). Because of the informative value of fibrosis stage it is worthwhile for clinicians to assess the speed of the fibrosis progression. In a large population we observed that fibrosis progression rate was not normally distributed with an asymmetrical distribution suggesting the presence of at least three populations: one population of "rapid fibrosers", a population of "intermediate fibrosers" and one population of "slow fibrosers" (5). Using the median fibrosis progression rate, and without treatment, the median expected time to cirrhosis was 30 years; 33% of patients had an expected median time to cirrhosis of less than 20 years and 31% will never progress to cirrhosis, or would do so in more than 50 years. There are no clear cut explanation to the individual susceptibility of developing liver fibrosis. However several factors have been clearly shown as associated with higher fibrosis progression rate: duration of infection, late age, male gender, consumption of alcohol and HIV coinfection with low CD4 count. For example, the estimated probability of progression per year for men aged 61-70 years was 300 times greater than that for men aged between 21 and 40 years and 10 times greater than that for women aged 61-70 years. Virus-associated factors such as genotype, viral load, quasi species are not associated with fibrosis.

Better knowledge of factors associated with individual susceptibility will allow a better definition of the patients' risk of developing fibrosis.

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Alcoholic and nonalcoholic steatohepatitis

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Chronic alcohol abuse may lead to two different types of liver injury. Most drinkers develop fatty liver, which by itself is a reversible alteration and has a low risk of progressing to liver cirrhosis. Approximately 20-40% of heavy drinkers, however, develop a special type of alcoholic liver disease, namely alcoholic steatohepatitis (ASH), which rapidly progresses to liver cirrhosis in most of the affected patients. ASH is characterized by the ballooning of hepatocytes, steatosis, hepatocellular necrosis and apoptosis, pericellular and perivenular fibrosis, inflammation with predominantly poly-

morphonuclear granulocytes, cholestasis and activation of Kupffer cells (1). Furthermore, a hallmark lesion seen in hepatocytes is the appearance of cytokeratin (CK)-containing cytoplasmic inclusions, termed Mallory bodies (MBs), which is accompanied by a disruption of the CK intermediate filament cytoskeleton (2-4). These alterations, however, are not specific for ethanol-induced toxic liver injury, but can also be found in patients without evidence of alcohol abuse. Occurrence of nonalcoholic steatohepatitis (NASH) is associated with obesity, noninsulin-dependent diabetes, intestinal bypass surgery, bacterial contamination of the small bowel, as well as with several drugs, such as amiodarone or perhexiline maleate, which are known to be inhibitors of mitochondrial β -oxidation (5). Identical hepatocytic alterations to that seen in human liver biopsies with ASH or NASH can be experimentally induced in mice by chronic intoxication with the porphyrogenic drugs griseofulvin (6) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DOG) (7). Analysis of the protein composition of MBs revealed that besides CKs also non-CK components, namely, the stress-inducible MM 120-1 antigen, a 62-65 kDa MB component recognized by the antibody SMI 31, and ubiquitin, which is a common constituent of a variety of cytoplasmic inclusions occurring in different chronic degenerative diseases, are present in MBs (8, 9). The role of these different components in MB formation as well as the relevance of MBs and the cytoskeletal alterations in the course of alcoholic hepatitis is still unclear.

To obtain further insight into the role of the different MB components and the alterations of the OK cytoskeleton in the pathogenesis of ASH and NASH, we investigated mice in which either of the two OK genes expressed in hepatocytes, namely CK8 and CK18, had been inactivated (10, 11). Since OK intermediate filaments are obligatory heteropolymers, no intermediate filaments can be formed in the absence of one of these two partner proteins resulting in hepatocytes devoid of a cytoplasmic OK network (12, 13). DDC intoxication of CK8^{-/-} mice showed a higher toxicity than in wild-type mice. After 3 months of intoxication seven out of 12 CK8^{-/-} animals had died, whereas all 13 K8^{+/+} mice survived. Analysis of metabolic alterations in these mice revealed that mice lacking CK8 develop a much severer porphyria than wild-type mice. Analysis of livers from DDC-fed CK8^{-/-} mice with double-label immunofluorescence microscopy showed that in the absence of CK8 no MBs were formed. Moreover, none of the non-OK MB components accumulated in these livers, indicating that OK is the core protein in MBs and that all other MB components either bind to or coassemble with CK. These *in vivo* data are in line with previously obtained *in vitro* data where we found that overexpression of CK by transient transfection of cells is sufficient to lead to induction of the MM 120-1 protein and to association of the MM 120-1 protein with cytokeratin aggregates, mimicking the initial phase of MB formation. To our surprise, DDC intoxication of mice with only one inactivated CK8 allele did not lead to alterations of the cytokeratin cytoskeleton nor to the appearance of MBs, although all other signs of DDC intoxication such as the loss of lamin B2 from the nuclear lamina, development of porphyria, proliferation of bile ductules were present. This different behavior of wild-type and heterozygous CK8 mutant mice has, therefore, to be attributed to the loss of one CK8 allele. To obtain more information on the functional consequences of the disruption of one CK8 allele, the mRNA concentrations of CK8 and OK18 were analyzed using a quantitative RT-PCR assay. DDC intoxication led to an approximately 5-fold overexpression of both CK8 and CK18 mRNAs in wild-type mice. In CK8^{+/+} mice, DDC caused a similar increase in the concentration of OK18