REVIEW

Molecular bases of hepatic fibrogenesis – genetic and therapeutical implications in chronic viral C hepatitis

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Abstract

Hepatitis C virus represents one of the major health problems of actual world, as almost 170 million of world population and 1 million persons in Romania are infected with HCV. Considering the increasing importance of HCV, it is imposed that we elucidate the molecular mechanisms, which are the base of hepatic fibrogenesis and potential targets for therapy, for diminishing progression to cirrhosis and avoid the appearance of complications. Activation of stellate cells is the main event in hepatic fibrosis. They also express almost all key components needed for the pathological degradation of matrix and that is why they play an important role not only in the production, but also in the degradation of the matrix. Recently, the worldwide research has also been oriented towards another type of cells with possible function in fibrogenesis and response to antiviral therapy: hepatic progenitor cells. The presence of hepatic progenitor cells in chronic C viral hepatitis is associated with severity of the disease, grade of fibrosis and the risk of hepatocarcinoma. Traditionally perceived as irreversible, reversibility of advanced fibrosis has been described recently in antiviral therapy trials for chronic C viral hepatitis. The favorable effect of interferon therapy on hepatic histology, including fibrosis, has been shown even in patients without sustained virusological response. During the last years, the advantages of the so-called support therapy using interferon have been demonstrated in patients with an increased rate in progression of fibrosis. Further research of the factors associated with progression of fibrosis will allow optimization of criteria for patient's antiviral therapy.

***Keywords*:** chronic viral C hepatitis, fibrogenesis, antiviral therapy.

Introduction

Hepatitis C virus (HCV) infection is one of the main health problems of contemporary society and it is probable that, in the absence of a vaccine, the epidemiological situation will worsen. WHO estimates that 170 million people (3% of world population) is infected with HCV and that every year there are 3–4 million new cases, so HCV infection is five times more common than HIV infection [1]. In Romanian population, there are approximately 1 million HCV infected persons.

The rate of chronic transformation of new cases is estimated at 80–90%, with the occurrence of hepatic cirrhosis after 15–20 years in 2–3% of cases annually and of hepatic cancer in 1.5–2% of cases per year, making the HCV infection the main indication for liver transplant and the second cause of hepatic carcinoma worldwide [2].

The impressive mortality and morbidity data once again emphasizes the importance of antiviral therapy in preventing the evolution of chronic hepatitis towards cirrhosis or hepatocellular carcinoma and the research of cellular mechanisms responsible for progression of fibrosis.

Pathogenic mechanisms in initiation and progression of hepatic fibrosis

The role of stellate cells

Hepatic fibrosis is a response of the healing process after injury and it is characterized by an accumulation of extracellular matrix ("scar") which follows chronic hepatic disease. Activation of stellate cells is the main event in hepatic fibrosis. In normal liver, the hepatic stellate cells are resting non-parenchimatous cells having the function of depositing vitamin A and maintaining the normal matrix.

The components of hepatic extracellular matrix include several types of structural and supporting molecules as non-collagenic glycoproteins, matrix growth factors, glucosaminoglycans, proteoglycans and matricellular proteins. In normal liver, the presence of collagen (type I, III, V and XI) is limited to the capsule, the areas around vessels and the portal space, with just rare fibrils containing type I and III, found in the subendothelial sector. In advanced fibrosis, the proportion of collagen fibers raises up to 3–10 times.

Stellate cells (formerly called lypocytes, Ito cells, perisinusoidal cells), are found in the perisinusoidal area, between the hepatocytes and sinusoidal endothelial

cells and are the main source of extracellular matrix in both normal and fibrotic liver. Activation of these cells is the key to the events in hepatic injury and consists in transition from the inactive, vitamin A rich cell to the high fibrogenic cell. Proliferation of stellate cells occurs in areas of significant hepatic destruction and is typically preceded by an influx of inflammatory cells and followed by accumulation of extracellular matrix.

Activation and perpetual proliferation of stellate cells

Activation of stellate cells is the central event in hepatic fibrosis and consists of two major phases: initiation (also known as preinflammatory stage) and perpetuation (inflammatory stage).

Kupffer cells infiltration and activation also plays an important role in the process and coincides with the appearance of stellate cell activation markers. Kupffer cells can stimulate synthesis of matrix, cell proliferation and release of retinoids from stellate cells through cytokines (especially TGF–beta 1) and lipidic peroxides.

The proliferation is attributed to TGF-alpha produced by Kupffer cells. TGF-beta derived from Kupffer cells significantly stimulates synthesis of extracellular matrix by stellate cells. Kupffer cells produce both antiinflammatory and pro-inflammatory cytokines, including interleukin 10 (IL–10) which regulates fibrogenesis in stellate cell cultures by decreasing synthesis of collagen and increasing production of collagenase. Another way in which Kupffer cells may influence stellate cells is by secretion of matrix metalloproteinase 9 (MMP–9, gelatinase B) which stimulates synthesis of collagen [3].

Degradation of matrix

Stellate cells express almost all key components needed for the pathological degradation of matrix and that is why they play an important part not only in the production, but also in the degradation of the matrix. An increasingly family of matrix metalloproteinases (calcium dependent enzymes) which specifically degrade collagens and non-collagenic structures has been identified.

Stellate cell activation markers in mioblast-like cells: alpha-smooth muscle actine (alpha-SMA), cyclooxygenase 2 (COX2), TNF-alpha, TGF-beta 1, fibroblast activation protein (FAP) are the base for identifying stellate cell activation by immunohisto-chemistry [4–7].

The intrahepatic expression of cyclooxygenase 2, matrix metalloproteinases 2 and 9 is significantly greater in patients with HCV than in patients with no hepatic disease and it progressively increases with fibrosis stage [8].

Hepatitis C virus replication releases factors that differently modulate the expression of stellate cells through key genes involved in fibrogenesis, increasing synthesis of procollagen I and III and lowering fibrinolytic activity of matrix proteinases. TGF-beta expression is several times greater in the case of HCV replication than in normal control [9].

Role of hepatic progenitor cells

In the last years, the research worldwide has also been oriented towards another type of cells with possible function in fibrogenesis and response to antiviral therapy: hepatic progenitor cells. Because of viral aggression, hepatic regeneration following hepatocyte necrosis takes place through two pathways: proliferation of mature hepatocytes and proliferation and differentiation of stem (progenitor) cells to hepatocytes [10]. The second pathway is activated by a combination of cytokines when proliferation of hepatocytes is stopped [11].

The presence of hepatic progenitor cells in chronic C viral hepatitis is associated with severity of the disease, grade of fibrosis and the risk of developing hepatocarcinoma. Additionally, recently has been demonstrated that treatment with alpha interferon in patients with chronic C viral hepatitis reduces the number of hepatic progenitor cells by modulating apoptosis, proliferation and differentiation [12].

Moreover, the presence of hepatic progenitor cells associated with the grade of fibrosis suggests a possible role of these cells in the development and progression of hepatic fibrosis. Recent studies show that activation of progenitor cells coexists with activation of Kupffer cells and stellate cells [13], and is correlated with portal fibrosis, suggesting an alternative pathway for regeneration [14]. The significant association between the expression of hepatic progenitor cells and the severity of disease and, more important, the response to antiviral therapy, proves that these cells can offer information about prognosis and evolution in patients with chronic C viral hepatitis [10]. Hepatic progenitor cells can be detected, like stellate cells, by immunohistochemistry.

Factors that influence progression of hepatic fibrosis

Progression of hepatic fibrosis is influenced by several factors, amongst which the role of structural proteins of HCV and viral replication has been recently demonstrated [9, 15,]. Other factors have been identified in epidemiological studies, factors that are correlated with histological severity: patient's age, duration of infection, alcohol ingestion, male gender and possibly body mass index [16, 17]. Amongst the histopathological factors involved, we mention necroinflammatory activity, grade of steatosis, grade of fibrosis (Figures 1–3) and iron load [18, 19].

The cytokine secreted as a response to cellular injury seem to have a central role in the pathogenesis of hepatic fibrosis. They are mediators for stellate cells, Kupffer cells but also for hepatic progenitor cells (for example, TGF-beta 1 is one of the most important cytokines involved in hepatic fibrogenesis, contributing to the activation of stellate cells with production of extracellular matrix). Stellate cells also produce antiinflamatory cytokines like IL-10, with antifibrotic activity, by lowering the synthesis of collagen and increasing the production of collagenase.

Completing the major antiinflammatory activity,

TNF-alpha decreases collagen synthesis and can support apoptosis of both inflammatory and fibrogenic cells. Therefore, cytokines are mediators, which, depending on their type and the cells they act upon, regulate fibrogenesis. The individual capacity of producing cytokines has a major genetic component. There is a marked difference between individuals regarding the capacity of producing cytokines after stimulation in vitro. This difference is attributed to polymorphism of regulatory regions or signal sequences in genes that code cytokines, as the involving of many genetic aspects has been demonstrated in the last years [20, 21].

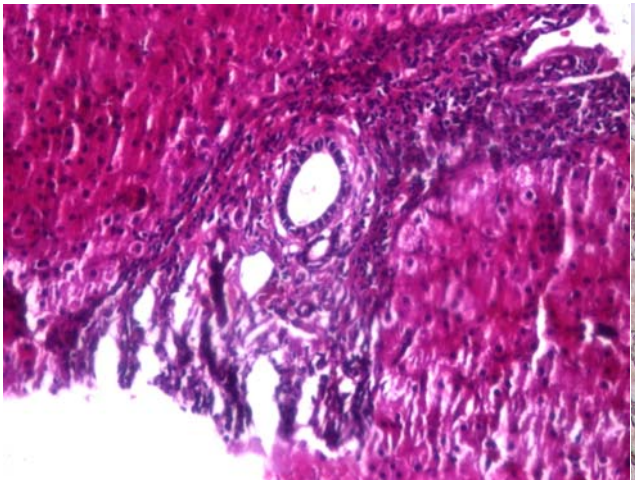

Figure 1 – ***Septal and portal fibrosis, biliar epithelium disorders (HE stain, ×100)***

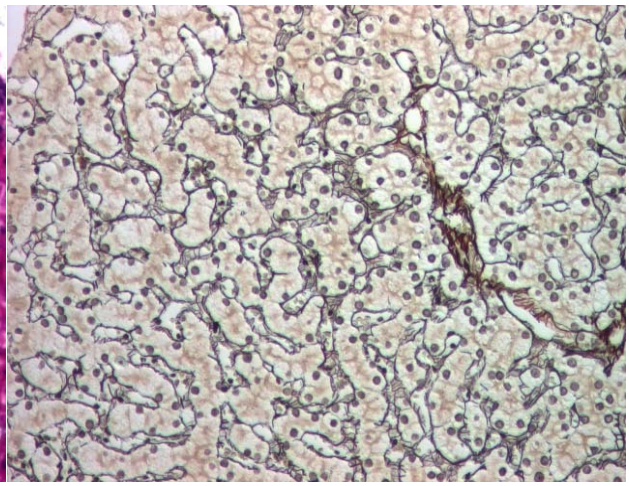

Figure 2 – ***Intralobular fibrosis (Gömöri stain, ×100)***

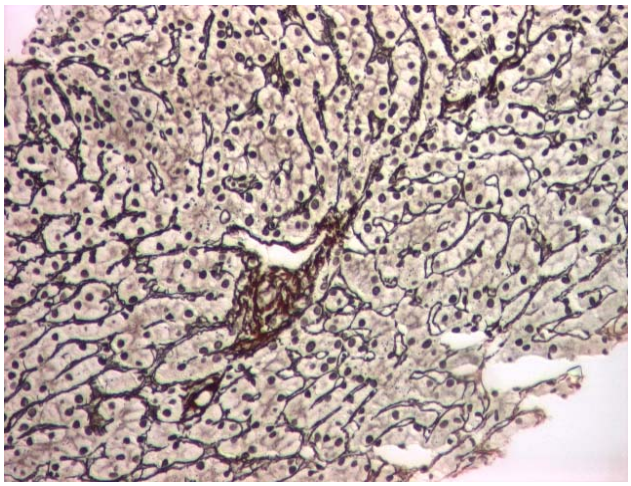

Figure 3 – ***Portal and short septal fibrosis (F2) (Gömöri stain, ×100)***

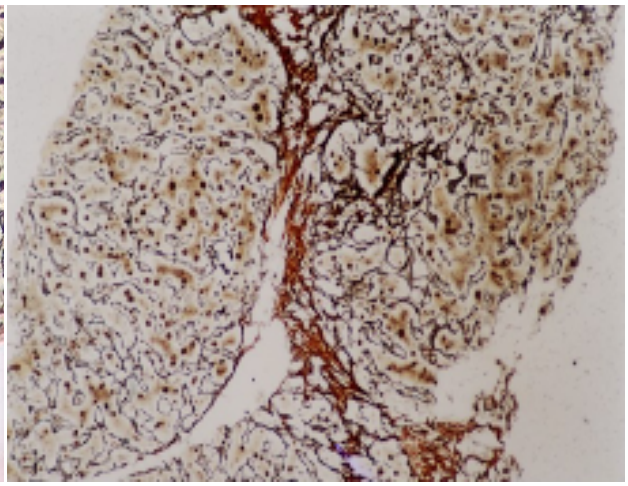

Figure 4 – ***Pseudonodules fibrosis (Gömöri stain, ×100)***

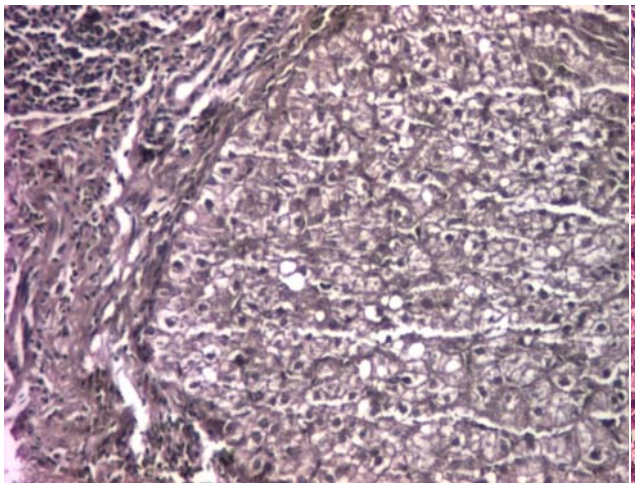

Figure 5 – ***Septal fibrosis (F2), vacuolar degeneration of hepatocytes, mild steatosis (Van Gieson stain, ×100)***

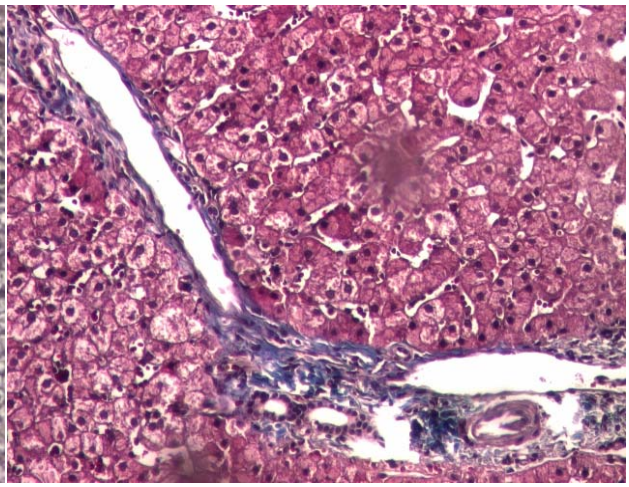

Figure 6 – ***Septal fibrosis and in porto-central bridges (F3) (Trichrome Masson stain, ×100)***

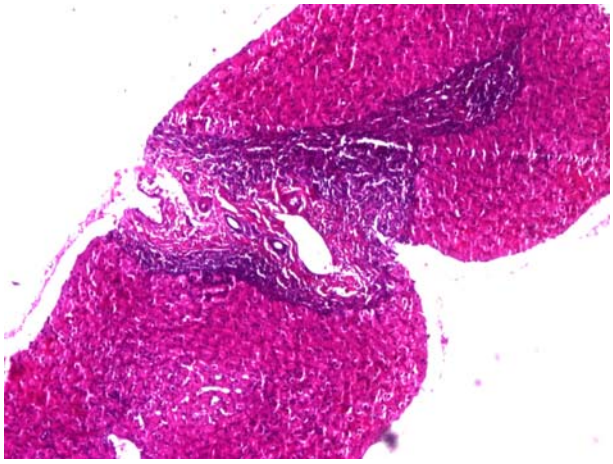

Figure 7 – *Fibrosis and inflammatory infiltrate in portal spaces and in septa (F2) (HE stain, ×40)*

Recently published studies have tried to identify the profile of gene expression depending on severity of fibrosis or the response to treatment [22]. In this way, there has been shown a different gene profile in non-responders, with major implications in the possible prediction of response to antiviral therapy.

In the same time, different gene profiles depending on stage of fibrosis have been found (Figures 4–7). The genes belong to the cytoskeleton (KRT 19, STMN2/SCG10), extracellular matrix (COL1A1, TIMP1), cytokine family/growth factors (CXCL6, CCR6, IL8, IL1A1, ITGA2, IL2) or have a major role in intercellular adhesion (CLDN4) [23].

Reversibility of fibrosis process

Traditionally perceived as irreversible, reversibility of advanced fibrosis has been described recently in antiviral therapy trials for chronic C viral hepatitis. Additionally, the significant improving of histological structure is associated with the decrease in number and activity of stellate cells [24, 25] and hepatic progenitor cells [10].

The favorable effect of interferon therapy on hepatic histology, including fibrosis, has been shown even in patients without sustained virusological response. Similarly, in clinical trials with combined antiviral therapy (interferon and ribavirine) the improving of histological scores was demonstrated [2].

Data gathered from 1509 patients included in three randomized clinical trials shows that sustained virusological response, duration of treatment and stage of fibrosis at the beginning were the predictive factors for reversibility of fibrosis [26].

In a recent meta-analysis of three randomized clinical trials, which included 1013 patients, Cammà C *et al.* found a significantly high improvement of the fibrosis score in patients that undergone treatment with peginterferon compared to those that received standard interferon [27]. Recent studies using current antiviral therapy reached a virusological response of approximately 60% [28]. In the patients that responded to antiviral therapy, there is a clear advantage, not only in slowing down progression of hepatic fibrosis, but also in reverse of fibrosis to an earlier stage [26].

During the last years, the advantages of the so-called *support therapy* using interferon have been demonstrated in patients with an increased rate in progression of fibrosis. In patients with acknowledged time of infection or that having two biopsies, one can estimate the rate in progression of fibrosis per year. Patients with advanced fibrosis, F2, F3, F4 or the ones with high rate in progression of fibrosis, over 0.2 progression of Metavir stage per year are candidates for support therapy with interferon [26].

In this moment, there is no efficient treatment for hepatic fibrosis. However, there is research that lead to generating antibodies against a surface peptide of stellate cells structure [29]. The efficiency of these antibodies is to be demonstrated.

Further research of the factors associated with progression of fibrosis will allow optimization of strategies to include patients in criteria for antiviral therapy. The significant improvement of histological structure at the end of the antiviral treatment was accompanied by a decrease in number and activity of stellate cells. These observations have important branches in understanding pathogenesis of hepatic fibrosis and in therapeutic approach. Development of hepatic fibrosis, particularly cirrhosis, is followed by severe complications and the patients' last hope is the liver transplant. The long waiting lists for transplant, the low number of organs available for transplant and the presence of comorbidity factors stop liver transplant from being a universal valid solution. Therefore, during the last years, the research was directed towards the study of stellate cells and hepatic progenitor cells, making them possible targets for potential antifibrotic therapy.

Note

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