# Pathophysiology of liver fibrosis and the methodological barriers to the development of anti-fibrogenic agents

Katrin Böttcher and Massimo Pinzani

University College London – Institute for Liver and Digestive Health, Royal Free Hospital,

NW3 2PF London, United Kingdom

**Address correspondence to:**

Professor Massimo Pinzani, MD, PhD, FRCP, FAASLD
UCL Institute for Liver and Digestive Health
Royal Free Hospital
Rowland Hill Road
London NW3 2PF
United Kingdom
m.pinzani@ucl.ac.uk

## Abstract

Liver fibrosis and cirrhosis resulting from long-standing liver damage represents a major health care burden worldwide. To date, there is no anti-fibrogenic agent available, making liver transplantation the only curative treatment for decompensated cirrhotic liver disease. Liver fibrosis can result from different underlying chronic liver disease, such as chronic viral infection, excessive alcohol consumption, fatty liver disease or autoimmune liver diseases. It is becoming increasingly recognised that as a result from different pathogenic mechanisms liver fibrosis must be considered as many different diseases for which individual treatment strategies need to be developed. Moreover, the pathogenic changes of both liver architecture and vascularisation in cirrhotic livers, as well as the lack of "true-to-life" *in vitro* models have impeded the development of an effective anti-fibrogenic drug. Thus, in order to identify an efficient anti-fibrogenic compound, novel *in-vitro* models mimicking the interplay between pro-fibrogenic cell populations, immune cells and, importantly, the extracellular matrix need to be developed.

**Key words:**

Liver fibrosis; chronic liver disease (CLD); hepatic stellate cells (HSC); anti-fibrotic drug; drug development; *in-vitro* models

## 1. General aspects/ introduction

Liver fibrosis is a chronic liver condition that develops as a result of a chronic wound healing response following long-standing liver injury. During hepatic fibrogenesis, the liver parenchyma undergoes fundamental remodelling characterized by progressive accumulation of fibrillar extracellular matrix (ECM) associated with nodular regeneration of the liver parenchyma. If untreated, liver fibrosis develops into cirrhosis and results in progressive loss of the normal liver function, which can lead to liver failure and death [1, 2].

In Europe, liver cirrhosis is the fourth most common cause of death with a prevalence of 76.3 per 100,000 aged over 25 in 2001 in the United Kingdom, and is more likely to occur in men [3]. The development of liver cirrhosis is driven by several different risk factors, the frequency of which varies regionally. Thus, in western countries excessive alcohol consumption, hepatitis C virus (HCV) infection and fatty liver disease are most common, whereas chronic hepatitis B virus (HBV) infection is the main risk factor in Asia [4, 5]. Furthermore, liver cirrhosis can evolve from a chronic immune-mediated damage in the context of autoimmune liver disease (AILD), such as primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH) [6-8]. Other less common risk factors include Wilson's disease (copper overload), haemochromatosis (iron overload) and $\alpha$1-antitrypsin deficiency, while some cases are cryptogenic [9, 10].

Although liver fibrosis has historically been considered as one disease, it has become clear that the pathophysiology of liver cirrhosis varies depending on the underlying aetiology, which has not only changed the perception of liver cirrhosis, but also created new challenges in treating cirrhosis.

Preventing the progression to cirrhosis and even attempting a regression of the fibrogenic process is based on treating the underlying cause of disease, as the progression of liver fibrosis, and even cirrhosis, can be attenuated when the harmful agent or stimulus is removed [11, 12]. Hence, antiviral treatment in HCV and HBV infection, immunosuppression in autoimmune hepatitis, abstinence from alcohol in alcoholic liver disease, weight loss and lifestyle change in fatty liver disease, venesection for haemochromatosis and copper chelating agents or zinc in Wilson's disease have been established as means to stabilize and possibly even reverse disease progression [10, 13-15]. Nevertheless, the possibility of approaching established fibrosis and even cirrhosis with an effective anti-fibrotic strategy would immensely change the prognosis and the overall management of patients with advanced liver fibrosis and cirrhosis. For this reason, extensive investments have been made in the past 20-30 years for the development of anti-fibrotic drugs exploring different therapeutic approaches and routes of drug delivery.

Importantly, despite the deeper knowledge of the pathophysiology and advances in treating liver cirrhosis, this condition still represents the main indication for over 5000 liver transplantations in Europe per year [4, 10], which is the only curative treatment for end-stage, decompensated liver cirrhosis at present. This is further aggravated by the fact that liver transplantation is not eligible to all cirrhotic patients and there is a severe lack of donor organs, stressing the need for novel and high impact therapeutic strategies. This article summarises the current knowledge on the mechanisms of liver fibrogenesis and attempts an analysis on the methodological barriers to the development of anti-fibrotic agents to be tested in preclinical studies.

## 2. Hepatic Fibrogenesis: general mechanisms

The development of liver fibrosis and subsequent cirrhosis is driven by ongoing liver injury through multiple mechanisms, and can be considered as an excessive wound healing response fuelled by a pathogenic vicious circle of hepatocyte necrosis, inflammation and excessive ECM deposition [1, 16]. Progression from healthy liver tissue to cirrhosis occurs after approximately 15-20 years of chronic hepatocellular damage [16], by when the cirrhotic liver contains up to six times more ECM than a normal liver [13]. Long-term chronic exposure to toxic agents such as hepatitis viruses, alcohol or bile acids can induce hepatocyte damage and apoptosis. In response, a repair reaction is triggered, which is characterized by ECM deposition and inflammation and results in liver fibrosis, when not only the exposure to toxic agents, but also the repair reaction is chronic [1]. The main ECM producing cell type in the liver are hepatic stellate cells (HSCs) which develop into hyper proliferative, ECM secreting myofibroblasts upon activation [1, 17]. Although HSCs are the main source of myofibroblasts in the liver [18, 19], other cell types contribute to the pool of fibrogenic myofibroblasts in liver disease. Portal myofibroblasts are located around bile ducts and play a role for the development of biliary fibrosis [20, 21]. Moreover, bone marrow derived myofibroblasts are thought to contribute to the development of liver fibrosis [22], although their contribution in murine fibrosis has shown to be minimal [23].

Activation of HSCs is stimulated by damaged and apoptotic hepatocytes through two main routes: release of damage-associated reactive oxygen species (ROS) and other fibrogenic mediators [24, 25] and recruitment of immune cells, which in turn mediate HSC activation and stimulate collagen secretion through release of cytokines and chemokines [26, 27]. Following the initial activation of HSCs, cytokines secreted by HSCs in an autocrine manner,

as well as immune cell derived cytokines, provide signals that maintain HSC activation and survival and the associated ECM deposition [17]. As a result, a vicious circle emerges, in which mutual stimulation between inflammatory and pro-fibrogenic cells drives hepatic fibrogenesis [28, 29].

Besides affecting the quantity of ECM, liver fibrosis also results in changes in the quality and topographic distribution of different ECM components. In the healthy liver, the ECM in the space of Disse, the space between endothelial cells and hepatocytes, mainly consists of collagen IV and VI. During fibrosis development, ECM is replaced by fibrillary collagens, such as collagen I and III, as well as fibronectin, leading to so-called capillarization of the sinusoids [30]. When fibrosis is established and chronic liver diseases has evolved from fibrosis to cirrhosis, major structural changes including extensive capillarisation of the liver sinusoids and formation of intrahepatic vascular shunts, as well as functional abnormalities, such as endothelial dysfunction, occur. Endothelial dysfunction results from decreased endothelial synthesis of vasodilators, such as nitric oxide, as well as increased secretion of vasoconstrictors, such as thromboxane A2 and endothelin [31, 32].

Such structural and functional changes result in the development of portal hypertension (PH), the major complication of liver cirrhosis, which in turn gives rise to other clinically relevant complications of cirrhosis, including ascites, variceal bleeding, hepatic encephalopathy and renal failure [1, 10]. Moreover, liver cirrhosis is the major risk factor for the development of hepatocellular carcinoma (HCC), as more than 80% of HCCs develop on a fibrotic or cirrhotic background [16, 33]. The high risk of HCC development represents a major healthcare issue, as HCC is the fifth most common solid tumour and the second leading cause for cancer deaths worldwide with a rising incidence in Europe and the United States of America [34, 35].

## 3. Cirrhosis or Cirrhoses?

Liver fibrosis can result from many different conditions, in which liver damage shows characteristic patterns of injury [2]. Along these lines, liver fibrosis shows different morphological patterns according to the underlying aetiology. Thus, viral hepatitis is associated with interface hepatitis and portal-central vein bridging fibrosis, whereas alcoholic fibrosis and non-alcoholic steatohepatitis (NASH) are characterized by perisinusoidal or pericellular fibrosis showing a so-called chicken wire pattern. In biliary cirrhosis, bile duct and portal myofibroblast proliferation result in the formation of portal-portal fibrotic septa [2, 36, 37]. Moreover, some pathophysiological mechanisms contributing to hepatic fibrogenesis are distinct between different aetiologies, whereas other mechanisms are shared across aetiologies, highlighting the need of individual concepts for the therapy of liver fibrosis and cirrhosis.

### 3.1 Alcoholic liver disease (ALD)

Resulting from long-standing excessive alcohol consumption, ALD can range from hepatic steatosis to acute alcoholic hepatitis to the development of liver fibrosis and cirrhosis on the basis of which HCC can develop [38]. Fibrosis development in ALD is driven by hepatocyte apoptosis and formation of ROS induced by the toxic effect of ethanol and its metabolite acetaldehyde [39, 40]. Moreover, several mechanisms specific to excessive alcohol intake stimulate HSC activation and thereby drive ECM deposition and inflammation. Thus, acetaldehyde can directly activate HSCs and stimulate collagen I expression [41]. Furthermore, bacteria derived lipopolysaccharide (LPS), which is translocated from the gut to

the liver due to increased gut permeability in ALD [42], can stimulate HSC activation directly via Toll-like-receptor (TLR) 4 ligation [43, 44]. Along these lines, LPS acts indirectly on HSC activation via stimulation of Kupffer cells, which in turn secrete HSC activating cytokines [45]. Ethanol furthermore inhibits the function of natural killer (NK) cells, which can contribute to fibrosis resolution through IFNγ secretion and killing of activated HSCs, thereby suppressing the anti-fibrotic effects of NK cells [46].

### 3.2 NAFLD/NASH

Non-alcoholic fatty liver disease (NAFLD) and its more severe form NASH occur in the context of the metabolic syndrome and are characterized by hepatic steatosis which can lead to the development of fibrosis and cirrhosis over time [47]. The formation of ROS and resulting oxidative stress induced by a mitochondrial overflow of free fatty acids is thought to be a critical factor in fibrosis development in NAFLD/NASH though several pathways. Oxidative stress hinders the replication of mature hepatocytes, thus leading to an accumulation of immature progenitor cells [48] originating from the Canals of Hering. Proliferation of such progenitor cells results in the formation of small ductules. This so-called ductular reaction has been linked to the development of fibrosis in NAFLD/NASH as the newly formed ductular cells secrete pro-inflammatory cytokines [49], and epithelial-mesenchymal transition of cholangiocytes to fibrogenic myofibroblasts can occur [50]. Moreover, hepatic steatosis is accompanied by an inflammatory reaction with elevated levels of pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα, mediated by activation of the Iκκ–β/NF-κB signalling pathway in the liver [51]. Free fatty acids can directly activate the Iκκ–β/NF-κB signalling pathway in

hepatocytes [52] and it has been shown that cytokine production by hepatocytes is a critical factor for the progression of steatosis to NASH [53].

### 3.3 Viral hepatitis

Both HBV and HCV are non-cytopathic viruses, thus liver damage is mediated by the host's immune system attempting to clear the virus [54]. However, virus-specific T cell typically become exhausted in viral hepatitis [55, 56], and are therefore unable to eliminate the virus, leading to chronic infection and inflammation which results in liver damage and fibrosis development [57]. As such exhausted virus-specific T cells are unable to mediate liver damage, necroinflammation in viral hepatitis is driven by secondary recruitment of mononuclear cells [58]. Moreover, some HCV proteins are able to stimulate the activation of pro-fibrogenic and pro-inflammatory properties of HSCs, thereby directly contributing to fibrogenesis [59]. In hepatocytes, HCV proteins induce the production of ROS and subsequent oxidative stress. Such ROS are thought to contribute to fibrogenesis both through direct damaging effects on hepatocytes and other cell types, as wells as by activation of hepatic stellate cells [60].

### 3.4 Autoimmune liver diseases

Autoimmune liver diseases (AILD) such as AIH, PBC and PSC are characterized by chronic inflammation which plays a pathogenic role for the development of fibrosis in these diseases. Immunologically, a loss of self-tolerance leads to autoimmune cell damage, and hepatic

inflammation is aggravated by aberrant recruitment of immune cells to the liver, as well as by an altered balance in both immune regulation and the response to foreign and self antigens [6, 61]. In the cholestatic autoimmune liver diseases PSC and PBC, intrahepatic accumulation of bile acids contributes to fibrogenesis through induction of hepatocyte and cholangiocyte apoptosis and necrosis [6, 62], as well as through activation of the bile acid/farnesoid X receptor (FXR). It has been demonstrated that a lack of FXR specifically inhibits cholestatic fibrogenesis [63], proposing the FXR axis as a novel therapeutic target in AILD. Moreover, it has been demonstrated that cholangiocytes play a role for fibrosis development in cholangiopathies. In particular, the cross-talk between proliferating cholangiocytes and portal fibroblasts, which are contributing to fibrosis development in PSC and PBC, has been highlighted as critical for fibrogenesis. Thus, cholangiocytes secrete a vast number of pro-fibrogenic effectors including pro-proliferative cytokines, chemokines and growth factors, which show an activating effect on portal myofibroblasts [64].

### 3.5 Aetiology-driven patterns of liver fibrosis development

Although cirrhosis is the common result of progressive fibrogenesis, there are distinct patterns of fibrotic development, related to the underlying disorders causing the fibrosis and depending on the origin of fibrogenic cells [2]. Biliary fibrosis, characterized by the co-proliferation of reactive bile ductules and periductular myofibroblast-like cells at the portal-parenchymal interface, tends to follow a portal to portal direction with the consequent formation of portal-portal septa surrounding liver nodules, where the central vein and its connections with the portal tract are preserved until late stages. In contrast, the chronic viral hepatitis characterized by portal-central (vein) bridging necrosis leads to the formation of

portal-central septa and to the rapid derangement of the vascular connections with the portal system (early portal hypertension). The so-called central to central (vein) form of fibrogenic evolution is in general secondary to venous outflow problems (e.g. chronic heart failure) and is characterized by the development of central to central septa and “reversed lobulation”. Finally, pericellular/perisinusoidal fibrosis is observed in alcoholic and metabolic liver diseases (e.g. NASH), in which the deposition of fibrillar matrix is concentrated around the sinusoids (capillarization) and around groups of hepatocytes (chicken-wire pattern). These different patterns of fibrogenic evolution are related to different factors and particularly: 1. the topographic localization of tissue damage, 2. the relative concentration of pro-fibrogenic factors, 3. the prevalent pro-fibrogenic mechanism(s) and 4. the origin of the pro-fibrogenic myofibroblast. In addition, these different patterns imply the participation of different cellular effectors of the fibrogenic process.

These striking differences in the development of liver fibrosis and the relative prevalent mechanisms typical of chronic liver diseases caused by different aetiologies represent a crucial issue when discussing the features of the proposed anti-fibrotic treatments and the development of biomarkers to be employed alongside the treatment to monitor its effectiveness.

**4. Chronic liver diseases: the actual need for anti-fibrotic strategies**

At present, no drugs with the specific indication “anti-fibrotic” are available in spite of over 30 years of active research in this field. To date, specific therapies for liver disease have primarily been aetiology-driven by eliminating or ameliorating the causative agent of chronic

liver disease (CLD). Recent examples include the introduction of anti-viral agents for hepatitis B and C virus infections, which have been fruitful in blocking chronic liver injury and thus progression of fibrosis, and even in reversing advanced fibrosis. Overall, the increasing success of antiviral treatments in blocking or reversing the fibrogenic progression of CLD has provided vital information about the natural history of fibrosis regression, and has established important principles and targets for anti-fibrotic drugs at least concerning fibrogenic disorders of the liver [65]. It is important to stress that despite the documented evidence of fibrosis and cirrhosis regression in animal models and the reabsorption of scar tissue following an effective causative treatment, the full reversibility of fibrosis in patients with advanced fibrosis and cirrhosis is still debated [66, 67].

Besides the obvious advantage of employing anti-fibrotic agents in CLD where there is no effective causative pharmacological treatment (basically all CLD with the exclusion of chronic HBV and HCV and to a certain extent autoimmune hepatitis), the use of anti-fibrotic agents would be particularly advantageous in CLD where the cause of damage has been eliminated only in a very advanced phase of the disease. This situation is typically represented by the elimination of HCV (sustained viral response, SVR) in patients with compensated or decompensated cirrhosis. Indeed, a key lesson emerging from several recent studies is that in compensated cirrhotic patients with clinically significant and severe PH, HCV clearance does not induce a significant reduction of PH and that cirrhosis, once advanced, may progress to decompensation even in absence of HCV replication. In biological terms, this can be explained by the relative autonomy acquired by the fibrogenic process beyond a certain level of development over decades, which is characterised by chronic fibro-inflammation and neo-angiogenesis. In particular, it is conceivable that, at this stage of the disease, two major

determinants may condition further clinical progression independently of the reduction of hepatocellular necrosis and inflammation induced by SVR. The first is represented by the remarkable hyperplasia of activated HSCs and myofibroblasts which is associated by a strong activation of anti-apoptotic pathways in these cells [68]. The second is due to the extensive changes in hepatic angioarchitecture as a consequence of neoangiogenesis and of the contraction of scar tissue leading to elevated tissue tension and are only minimally affected by the reduction of necroinflammation following SVR [69, 70].

**5. Methodological barriers to the development of anti-fibrotic agents for liver fibrosis**

The anatomical changes typical of the progression of chronic fibrogenic disorders of the liver and the progressive alteration, both in terms of topography and biochemistry, of the structure of the hepatic ECM stroma, represent the main barriers for drug delivery to target cells. Indeed, in advanced stages of the disease, irrespective of the aetiology of CLD, scar tissue is characterized by extensive collagen cross-linking, abundance of elastin, dense acellular/paucicellular ECM, and decreased expression and/or activity of specific metalloproteinases [71, 72]. In these conditions, independently of the cellular or molecular target of the drug under evaluation, the biochemical changes of the ECM and the vascular disconnection of large areas of the fibrotic tissue constitute a major and almost impenetrable physical barrier and definitely need to be considered when the end point of a proposed treatment is the reduction of fibrosis in a cirrhotic liver (if not the reversal of cirrhosis!).

Another important aspect to be considered when addressing the reversibility of fibrosis and cirrhosis in human CLD (as opposed to animal models of liver fibrosis) is the long term

activation of HSC and their hyperplasia. Indeed, evidence obtained in studies employing freshly isolated vs. activated human HSC and human tissue samples indicates that established fibrotic tissue is characterized by a progressive resistance to apoptosis of HSC, leaving a critical mass of pro-fibrogenic cells refractory to reversion back to a quiescent state [68].

In any case, a crucial point to be always taken into account is the targeted pro-fibrogenic mechanism. Since the knowledge of the pathways possibly contributing to tissue scarring has grown exponentially as consequence of over 30 years of active research, there are several possible scenarios to be considered. The first scenario is provided by a treatment targeting a single cytokine shown to play a key pro-inflammatory and/or pro-fibrogenic role. In this case, the target (i.e. the cytokine itself or its receptors) is validated in a specific $^{-}/^{-}$ animal model where the effect is almost inevitably an “all or nothing” effect. However, there is a very large probability that in the context of human fibrogenic diseases, developing in decades rather than weeks, the target cytokine is part of a pleiotropic network of mediators that will ultimately dilute the effects of its blockade. Indeed, the current way of thinking and validating potential targets constitutes a methodological barrier for the development of effective anti-fibrogenic strategies per se. Along these lines, expectations on the efficacy of anti-fibrogenic agents are based on the adequacy of the targets identified as key mechanisms of fibrogenesis and on the results of *in vitro* studies and studies performed in experimental animal models. Accordingly, most of the failures of putative anti-fibrotic agents observed in the treatment of human disease are related to the large inadequacy of the pre-clinical platforms. In this context, the molecular mechanisms of liver fibrogenesis are largely investigated in cell culture of HSC (often murine and often immortalized cell lines rather than human primary cells) according to a “cell-centric” approach in which cell functions are analysed in the artificial

context of a 2D monolayer cell culture on plastic dishes. This prevents to take into consideration the key relationship between the fibrogenic cell itself and the surrounding diseased tissue microenvironment and in particular its 3D extracellular matrix biochemical structure and stiffness. Attempts to improve this methodological issue are currently in progress as described in detail in the article by Rombouts and Mazza published in this issue of Advanced Drug Delivery Reviews (add reference).

**Acknowledgements**

K.B. is funded by the European Association for the Study of the Liver (EASL)

**References**

[1] M. Pinzani, Pathophysiology of Liver Fibrosis, Dig Dis, 33 (2015) 492-497.
[2] M. Pinzani, K. Rombouts, Liver fibrosis: from the bench to clinical targets, Digestive and Liver Disease, 36 (2004) 231-242.
[3] K.M. Fleming, G.P. Aithal, M. Solaymani-Dodaran, T.R. Card, J. West, Incidence and prevalence of cirrhosis in the United Kingdom, 1992–2001: A general population-based study, Journal of Hepatology, 49 (2008) 732-738.
[4] M. Blachier, H. Leleu, M. Peck-Radosavljevic, D.-C. Valla, F. Roudot-Thoraval, The burden of liver disease in Europe: A review of available epidemiological data, Journal of Hepatology, 58 (2013) 593-608.

[5] D. Ganem , A.M. Prince Hepatitis B Virus Infection — Natural History and Clinical Consequences, New England Journal of Medicine, 350 (2004) 1118-1129.
[6] K. Arndtz, G.M. Hirschfield, The Pathogenesis of Autoimmune Liver Disease, Dig Dis, 34 (2016) 327-333.
[7] E. Liaskou, G.M. Hirschfield, M.E. Gershwin, Mechanisms of tissue injury in autoimmune liver diseases, Seminars in immunopathology, 36 (2014) 553-568.
[8] R. Liberal, C.R. Grant, Cirrhosis and autoimmune liver disease: Current understanding, World J Hepatol, 8 (2016) 1157-1168.
[9] M. Pinzani, M. Rosselli, M. Zuckermann, Liver cirrhosis, Best Practice & Research Clinical Gastroenterology, 25 (2011) 281-290.
[10] E.A. Tsochatzis, J. Bosch, A.K. Burroughs, Liver cirrhosis, The Lancet, 383 1749-1761.
[11] R. Perez-Tamayo, Cirrhosis of the liver: a reversible disease?, Pathol Annu, 14 Pt 2 (1979) 183-213.
[12] E.L. Ellis, D.A. Mann, Clinical evidence for the regression of liver fibrosis, Journal of Hepatology, 56 (2012) 1171-1180.
[13] M.J. Arthur, D.A. Mann, J.P. Iredale, Tissue inhibitors of metalloproteinases, hepatic stellate cells and liver fibrosis, J Gastroenterol Hepatol, 13 Suppl (1998) S33-38.
[14] A. Pares, J. Caballeria, M. Bruguera, M. Torres, J. Rodes, Histological course of alcoholic hepatitis. Influence of abstinence, sex and extent of hepatic damage, J Hepatol, 2 (1986) 33-42.
[15] J.B. Dixon, P.S. Bhathal, N.R. Hughes, P.E. O'Brien, Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss, Hepatology, 39 (2004) 1647-1654.
[16] R. Bataller, D.A. Brenner, Liver fibrosis, Journal of Clinical Investigation, 115 (2005) 209-218.
[17] S.L. Friedman, Hepatic Stellate Cells: Protean, Multifunctional, and Enigmatic Cells of the Liver, Physiological Reviews, 88 (2008) 125-172.
[18] I. Mederacke, C.C. Hsu, J.S. Troeger, P. Huebener, X. Mu, D.H. Dapito, J.-P. Pradere, R.F. Schwabe, Fate-tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its etiology, Nature communications, 4 (2013) 2823-2823.
[19] S.L. Friedman, Evolving challenges in hepatic fibrosis, Nat Rev Gastroenterol Hepatol, 7 (2010) 425-436.
[20] E. Novo, L. Valfrè di Bonzo, S. Cannito, S. Colombatto, M. Parola, Hepatic myofibroblasts: A heterogeneous population of multifunctional cells in liver fibrogenesis, The International Journal of Biochemistry & Cell Biology, 41 (2009) 2089-2093.
[21] M. Parola, F. Marra, M. Pinzani, Myofibroblast – like cells and liver fibrogenesis: Emerging concepts in a rapidly moving scenario, Molecular Aspects of Medicine, 29 (2008) 58-66.
[22] F.P. Russo, M.R. Alison, B.W. Bigger, E. Amofah, A. Florou, F. Amin, G. Bou–Gharios, R. Jeffery, J.P. Iredale, S.J. Forbes, The Bone Marrow Functionally Contributes to Liver Fibrosis, Gastroenterology, 130 (2006) 1807-1821.
[23] R. Higashiyama, T. Moro, S. Nakao, K. Mikami, H. Fukumitsu, Y. Ueda, K. Ikeda, E. Adachi, G. Bou–Gharios, I. Okazaki, Y. Inagaki, Negligible Contribution of Bone Marrow-Derived Cells to Collagen Production During Hepatic Fibrogenesis in Mice, Gastroenterology, 137 (2009) 1459-1466.e1451.
[24] A. Canbay, P. Taimr, N. Torok, H. Higuchi, S. Friedman, G.J. Gores, Apoptotic body engulfment by a human stellate cell line is profibrogenic, Laboratory investigation; a journal of technical methods and pathology, 83 (2003) 655-663.
[25] A. Canbay, S. Friedman, G.J. Gores, Apoptosis: the nexus of liver injury and fibrosis, Hepatology, 39 (2004) 273-278.
[26] A. Casini, E. Ceni, R. Salzano, P. Biondi, M. Parola, A. Galli, M. Foschi, A. Caligiuri, M. Pinzani, C. Surrenti, Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: Role of nitric oxide, Hepatology, 25 (1997) 361-367.
[27] T. Luedde, N. Kaplowitz, R.F. Schwabe, Cell death and cell death responses in liver disease: mechanisms and clinical relevance, Gastroenterology, 147 (2014) 765-783.e764.

[28] J.J. Maher, Interactions between hepatic stellate cells and the immune system, Seminars in liver disease, 21 (2001) 417-426.
[29] E. Seki, R.F. Schwabe, Hepatic Inflammation and Fibrosis: Functional Links and Key Pathways, Hepatology, (2014).
[30] A.M. Gressner, M.G. Bachem, Molecular Mechanisms of Liver Fibrogenesis – A Homage to the Role of Activated Fat-Storing Cells, Digestion, 56 (1995) 335-346.
[31] M. Pinzani, S. Milani, R. De Franco, C. Grappone, A. Caligiuri, A. Gentilini, C. Tosti-Guerra, M. Maggi, P. Failli, C. Ruocco, P. Gentilini, Endothelin 1 is overexpressed in human cirrhotic liver and exerts multiple effects on activated hepatic stellate cells, Gastroenterology, 110 (1996) 534-548.
[32] J.-C. García-Pagán, J. Gracia-Sancho, J. Bosch, Functional aspects on the pathophysiology of portal hypertension in cirrhosis, Journal of Hepatology, 57 (2012) 458-461.
[33] S.S. Thorgeirsson, J.W. Grisham, Molecular pathogenesis of human hepatocellular carcinoma, Nat Genet, 31 (2002) 339-346.
[34] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012, International Journal of Cancer, 136 (2015) E359-E386.
[35] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA: A Cancer Journal for Clinicians, 61 (2011) 69-90.
[36] M. Pinzani, K. Rombouts, S. Colagrande, Fibrosis in chronic liver diseases: diagnosis and management, Journal of Hepatology, 42 (2005) S22-S36.
[37] V. Hernandez-Gea, S.L. Friedman, Pathogenesis of liver fibrosis, Annu Rev Pathol, 6 (2011) 425-456.
[38] A. Louvet, P. Mathurin, Alcoholic liver disease: mechanisms of injury and targeted treatment, Nat Rev Gastroenterol Hepatol, 12 (2015) 231-242.
[39] M. Setshedi, J.R. Wands, S.M. de la Monte, Acetaldehyde adducts in alcoholic liver disease, Oxidative Medicine and Cellular Longevity, 3 (2010) 178-185.
[40] A.E. Feldstein, G.J. Gores, Apoptosis in alcoholic and nonalcoholic steatohepatitis, Front Biosci, 10 (2005) 3093-3099.
[41] T. Mello, E. Ceni, C. Surrenti, A. Galli, Alcohol induced hepatic fibrosis: role of acetaldehyde, Mol Aspects Med, 29 (2008) 17-21.
[42] R. Rao, Endotoxemia and gut barrier dysfunction in alcoholic liver disease, Hepatology, 50 (2009) 638-644.
[43] P. Brun, I. Castagliuolo, M. Pinzani, G. Palù, D. Martines, Exposure to bacterial cell wall products triggers an inflammatory phenotype in hepatic stellate cells, American Journal of Physiology - Gastrointestinal and Liver Physiology, 289 (2005) G571-G578.
[44] E. Seki, S. De Minicis, C.H. Osterreicher, J. Kluwe, Y. Osawa, D.A. Brenner, R.F. Schwabe, TLR4 enhances TGF-beta signaling and hepatic fibrosis, Nat Med, 13 (2007) 1324-1332.
[45] V. Purohit, D.A. Brenner, Mechanisms of alcohol-induced hepatic fibrosis: A summary of the Ron Thurman Symposium, Hepatology, 43 (2006) 872-878.
[46] W.I. Jeong, O. Park, B. Gao, Abrogation of the antifibrotic effects of natural killer cells/interferon-gamma contributes to alcohol acceleration of liver fibrosis, Gastroenterology, 134 (2008) 248-258.
[47] M. Rosselli, S. Lotersztajn, F. Vizzutti, U. Arena, M. Pinzani, F. Marra, The metabolic syndrome and chronic liver disease, Current pharmaceutical design, 20 (2014) 5010-5024.
[48] T. Roskams, S.Q. Yang, A. Koteish, A. Durnez, R. DeVos, X. Huang, R. Achten, C. Verslype, A.M. Diehl, Oxidative Stress and Oval Cell Accumulation in Mice and Humans with Alcoholic and Nonalcoholic Fatty Liver Disease, The American journal of pathology, 163 (2003) 1301-1311.
[49] G. Svegliati-Baroni, S. De Minicis, M. Marzioni, Hepatic fibrogenesis in response to chronic liver injury: novel insights on the role of cell-to-cell interaction and transition, Liver International, 28 (2008) 1052-1064.
[50] W.K. Syn, Y. Jung, A. Omenetti, M. Abdelmalek, C.D. Guy, L. Yang, J. Wang, R.P. Witek, C.M. Fearing, T.A. Pereira, V. Teaberry, S.S. Choi, J. Conde–Vancells, G.F. Karaca, A.M. Diehl, Hedgehog-

Mediated Epithelial-to-Mesenchymal Transition and Fibrogenic Repair in Nonalcoholic Fatty Liver Disease, Gastroenterology, 137 (2009) 1478-1488.e1478.
[51] D. Cai, M. Yuan, D.F. Frantz, P.A. Melendez, L. Hansen, J. Lee, S.E. Shoelson, Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB, Nat Med, 11 (2005) 183-190.
[52] A.E. Feldstein, N.W. Werneburg, A. Canbay, M.E. Guicciardi, S.F. Bronk, R. Rydzewski, L.J. Burgart, G.J. Gores, Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway, Hepatology, 40 (2004) 185-194.
[53] C.P. Day, From fat to inflammation, Gastroenterology, 130 (2006) 207-210.
[54] L.G. Guidotti, F.V. Chisari, Immunobiology and pathogenesis of viral hepatitis, Annu Rev Pathol, 1 (2006) 23-61.
[55] C. Boni, P. Fisicaro, C. Valdatta, B. Amadei, P. Di Vincenzo, T. Giuberti, D. Laccabue, A. Zerbini, A. Cavalli, G. Missale, A. Bertoletti, C. Ferrari, Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection, J Virol, 81 (2007) 4215-4225.
[56] A. Schurich, P. Khanna, A.R. Lopes, K.J. Han, D. Peppa, L. Micco, G. Nebbia, P.T. Kennedy, A.M. Geretti, G. Dusheiko, M.K. Maini, Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection, Hepatology, 53 (2011) 1494-1503.
[57] U. Protzer, M.K. Maini, P.A. Knolle, Living in the liver: hepatic infections, Nature reviews. Immunology, 12 (2012) 201-213.
[58] B. Rehermann, Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells, Nature Medicine, 19 (2013) 859-868.
[59] R. Bataller, Y.H. Paik, J.N. Lindquist, J.J. Lemasters, D.A. Brenner, Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells, Gastroenterology, 126 (2004) 529-540.
[60] J. Choi, J.H. Ou, Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus, American journal of physiology. Gastrointestinal and liver physiology, 290 (2006) G847-851.
[61] P.J. Trivedi, D.H. Adams, Gut–liver immunity, Journal of Hepatology, 64 (2016) 1187-1189.
[62] A.F. Hofmann, L.R. Hagey, Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics, Cellular and molecular life sciences : CMLS, 65 (2008) 2461-2483.
[63] P. Fickert, A. Fuchsbichler, T. Moustafa, M. Wagner, G. Zollner, E. Halilbasic, U. Stoger, M. Arrese, M. Pizarro, N. Solis, G. Carrasco, A. Caligiuri, M. Sombetzki, E. Reisinger, O. Tsybrovskyy, K. Zatloukal, H. Denk, H. Jaeschke, M. Pinzani, M. Trauner, Farnesoid X receptor critically determines the fibrotic response in mice but is expressed to a low extent in human hepatic stellate cells and periductal myofibroblasts, The American journal of pathology, 175 (2009) 2392-2405.
[64] M. Penz-Osterreicher, C.H. Osterreicher, M. Trauner, Fibrosis in autoimmune and cholestatic liver disease, Best Pract Res Clin Gastroenterol, 25 (2011) 245-258.
[65] M. Pinzani, Liver Fibrosis in the Post-HCV Era, Seminars in liver disease, 35 (2015) 157-165.
[66] S.L. Friedman, Reversibility of hepatic fibrosis and cirrhosis[mdash]is it all hype?, Nat Clin Pract Gastroenterol Hepatol, 4 (2007) 236-237.
[67] M. Pinzani, J. Macias-Barragan, Update on the pathophysiology of liver fibrosis, Expert Rev Gastroenterol Hepatol, 4 (2010) 459-472.
[68] E. Novo, F. Marra, E. Zamara, L. Valfre di Bonzo, L. Monitillo, S. Cannito, I. Petrai, A. Mazzocca, A. Bonacchi, R.S. De Franco, S. Colombatto, R. Autelli, M. Pinzani, M. Parola, Overexpression of Bcl-2 by activated human hepatic stellate cells: resistance to apoptosis as a mechanism of progressive hepatic fibrogenesis in humans, Gut, 55 (2006) 1174-1182.
[69] M. Pinzani, F. Vizzutti, Fibrosis and Cirrhosis Reversibility: Clinical Features and Implications, Clinics in Liver Disease, 12 (2008) 901-913.
[70] M. Fernandez, D. Semela, J. Bruix, I. Colle, M. Pinzani, J. Bosch, Angiogenesis in liver disease, J Hepatol, 50 (2009) 604-620.

[71] A. Hayasaka, S. Ilda, N. Suzuki, F. Kondo, M. Miyazaki, H. Yonemitsu, Pyridinoline collagen cross-links in patients with chronic viral hepatitis and cirrhosis, J Hepatol, 24 (1996) 692-698.
[72] R. Issa, X. Zhou, C.M. Constandinou, J. Fallowfield, H. Millward-Sadler, M.D. Gaca, E. Sands, I. Suliman, N. Trim, A. Knorr, M.J. Arthur, R.C. Benyon, J.P. Iredale, Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking, Gastroenterology, 126 (2004) 1795-1808.