HCV and Hepatic Extracellular Matrix

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Chronic infection by the hepatitis C virus (HCV) is a major cause of liver diseases, predisposing to fibrosis and hepatocellular carcinoma. Liver fibrosis is characterized by an overly abundant accumulation of components of the hepatic extracellular matrix, such as collagen and elastin, with consequences on the properties of this microenvironment and cancer initiation and growth. This review will provide an update on mechanistic concepts of HCV-related liver fibrosis/cirrhosis and early stages of carcinogenesis, with a dissection of the molecular details of the cross-talk during disease progression between hepatocytes, the extracellular matrix and hepatic stellate cells.

liver fibrosis | cirrhosis | chronic hepatitis C | carcinogenesis | extracellular matrix

1. Introduction

A recent report from the International Agency for Research on Cancer states that 15% of new cancer cases in 2012 were attributable to carcinogenic infections [1], caused by oncogenic viruses: human papillomavirus for cervical carcinoma, Epstein–Barr virus for Burkitt's and Hodgkin's lymphomas and nasopharynx carcinoma, and hepatitis B and C viruses (HBV and HCV, respectively) for hepatocellular carcinoma (HCC). Of note, 73% of HCC cases are attributable to HBV and HCV [1]. It is the fifth most common cancer worldwide and the second leading cause of cancer death. Its prognosis is poor, with a 5-year survival of only 18% [2]; moreover, diagnosis often occurs late, and curative therapy is not available. Over decades, chronic inflammation and oxidative stress induced by causative agents lead to chronic hepatic injury, with excessive wound healing and deposition of connective tissue (fibrosis), and disruption of hepatic architecture and function, with proliferation of regenerating hepatocytes (cirrhosis), eventually leading to chromosomal aberrations and malignant transformation of proliferating hepatocytes (HCC) [3]. However, HBV and HCV exhibit different pathogenesis and carcinogenic properties, recapitulated in Table 1. HCV displays a predominantly cytoplasmic life cycle, which renders it more likely to drive carcinogenesis through the alteration of cell signaling and metabolism and modulation of immune responses. These processes fuel chronic inflammation, oxidative stress, and repair mechanisms underlying liver fibrosis, cirrhosis, and HCC. Nevertheless, some HCV proteins have been shown to alter cell cycle checkpoint machinery such as the retinoblastoma protein Rb and the mitotic spindle [4]. With the discovery of direct-acting antivirals, HCV can be eliminated, and 95 to 99% of chronically infected patients can be considered cured [5]. Favorable outcomes of infection are now also obtained for cirrhotic and cancer patients.

Table 1. Main differences between HBV and HCV pathogenesis.

Virus	HBV	HCV
Viral family	*Hepadnaviridae*	*Flaviviridae*
Genome	DNA and cccDNA	RNA
Life cycle	Genome integration, expression of HBx protein, insertional activation of cellular oncogenes, cccDNA (minichromosome)	Exclusively cytoplasmic
Persistence	Nucleus-located cccDNA	Chronic inflammation, oxidative stress, alterations in cellular signaling and metabolism

Approximately 85% of HCV-infected individuals develop chronic hepatitis C. At present, ≈80 million individuals are chronically infected worldwide [1]. Patients with chronic hepatitis C are at risk of increased fibrosis progression, with subsequent complications of cirrhosis and HCC [6][7]. Based on the natural history of chronic hepatitis C, at least 30% will develop liver fibrosis, 7–18% will develop cirrhosis, and 1–5% HCC within 20–30 years [8]. A projection of the World Health Organization estimates that more than 1 million patients will die from liver cancer in 2030 [2].

After years of interferon-based therapies, the introduction of new antivirals directly targeting HCV replication (direct-acting antivirals (DAAs)) and achieving sustained virological response (SVR) in more than 95% of treated patients raised great hopes of a marked reduction in HCC occurrence and recurrence in patients with a history of previous liver cancer treated surgically. However, recent clinical observations report somewhat conflicting data [9]. SVR induced by anti-HCV therapies based upon interferon or upon DAAs may result in distinct post-SVR HCC risk [10]. HCV may lead to irreversible changes in cellular signaling (epigenetic events [11], imprinting), and recent data tend to indicate that chronic hepatitis C durably disrupts the balance of inflammatory mediators, even after HCV clearance [12]. These features could underlie a residual risk of carcinogenesis after viral clearance [11]. For example, a variant in the core protein of HCV genotype 1b is associated with increased HCC incidence post SVR [13]. A more recent study pointed at sustained oncogenic transcriptomic profiles in liver tissues after HCV eradication with DAAs [14]. Among them, increased serum levels of CYR61 could be a possible biomarker of HCC post-SVR [14] (see Section 3.2.4). Thus, SVR is a virological cure but is not necessarily translated into a cure from risks of liver disease, particularly for patients with cirrhotic-stage fibrosis.

Contamination with HCV occurs from a breach through the blood circulation, from which the virus is transported to the liver, where its target cells are hepatocytes. Virions are composed of three structural proteins: the capsid or

core protein, which compacts the viral genome, and the envelope glycoproteins E1 and E2, which permit viral entry through recognition of several surface receptors. HCV replication, involving nonstructural viral proteins (NS2, 3, 4A, 4B, 5A, 5B) is restricted to the cytoplasm of infected cells, where it engages the endoplasmic reticulum (ER) and lipid droplets. Unlike other carcinogenic viruses, HCV entirely replicates outside the nucleus of hepatocytes, and no latency or persistence factor is synthesized during its life cycle [15]. The deleterious effects of chronic hepatitis C are therefore anticipated to occur through a subtle interplay between viral determinants and the liver microenvironment in which the virus propagates. Studying HCV pathogenesis thus implies the thorough study of cellular and tissular alterations induced throughout chronic infection.

2. Main Actors of Liver Fibrosis

In the human liver, 80% of cells are hepatocytes. These epithelial cells are derived from the bipotential progenitor cells called hepatoblasts or hepatic progenitor cells (HPCs), also capable of differentiation toward cholangiocytes or bile duct cells, which delineate bile canaliculi [16]. Other cell types are hepatic stellate cells (HSCs), acting as reservoirs of vitamin A; endothelial cells, forming liver capillary sinusoids; and liver-resident macrophages (Kupffer cells). These macrophages are cells of great plasticity. Liver injury triggers their activation, leading to inflammatory cytokine and chemokine release, which fuels inflammation and fibrogenesis. However, if liver injury ceases, they switch their phenotype toward reparative phagocytes under specific signals, thereby promoting tissue repair and regression of fibrosis [17]. We will nevertheless only focus on hepatocytes and HSCs, as these cells produce components of the hepatic ECM, and hepatocytes are the targets of HCV infection. Nutrients, molecules from the hepatic microenvironment, and substances to be transported to the bile arrive from sinusoid capillaries, forming a fenestrated endothelium. The region between blood capillary sinusoids and hepatocytes is the space of Disse, containing HSCs and filled by the hepatic ECM. This space, part of the liver connective tissue [18], is an active zone of exchange between blood and hepatocytes.

In normal liver, HSCs are quiescent and exhibit a spindle-like shape; their most characteristic feature is the storage of retinoids in intracellular droplets [19]. HSCs also play a major role in liver development and regeneration by expressing and secreting hepatocyte mitogens such as hepatocyte growth factor (HGF) or epidermal growth factor (EGF) [15]. In injured liver, HSCs become activated, with a continuum of changes in gene expression during activation. Activated HSCs migrate and accumulate at the sites of tissue repair, secreting large amounts of ECM, mainly type I collagen fibrils that cross-link and deposit in the space of Disse, matrix metalloproteases (MMPs), and their inhibitors tissue inhibitors of MMPs (TIMPs). This contributes to the regulation of ECM remodeling. They also differentiate into myofibroblast-like cells expressing α-smooth muscle actin (α-SMA) [20]. Loss of retinoids and lipid droplets is concomitant to a de novo expression of receptors of profibrotic and chemotactic factors.

The hepatic ECM, as other ECM, can be subdivided into interstitial matrix and basement membrane. Liver ECM forms a very limited compartment of low density within the normal liver [21], comprising less than 3% of the relative area on a tissue section and approximately 0.5% of the wet weight [22]. It is of major importance in liver physiology through its scaffolding effect and roles in biological functions such as cell proliferation, migration, and differentiation. Liver ECM proteins are mostly detected in the Glisson capsule (the connective tissue surrounding

the liver), portal tracks, central veins, and in the subendothelial space of Disse. Collagens, fibronectin, laminins, proteoglycans, and matricellular proteins (such as thrombospondins, tenascins, and osteopontin) are the main ECM components in the normal liver. Fibrillar collagens type I, III, and V are mostly interstitial, in the portal and central regions. They are produced by activated HSCs and emanate from the cross-linking of collagen fibrils by lysyl oxidases (LOX) to form an insoluble scaffold, which helps support tissue structure [23][24]. The network-forming collagen IV is highly present in basement membranes. Adhesive glycoproteins such as fibronectin and tenascins are detected in the subcapsular connective tissue, septa, and portal areas, and fibronectin is the main ECM component in Disse's space in normal liver [25]. Proteoglycans (PGs, e.g., lumican and fibromodulin) act as "space fillers" of the ECM and function in the assembly of collagen fibrils; they are formed by a core protein onto which several glycosaminoglycan (GAG) chains are covalently attached (heparin, heparan, dermatan, keratan, and chondroitin sulfate) [26]. Low amounts of elastin are also present in this interstitial matrix, increasing in diseased liver [27]. The basement membrane of the liver ensures a scaffold for the attachment of hepatocytes and endothelial cells, and its loose structure allows for the rapid diffusion of small molecules. It is a prominent reservoir of angiogenic growth factors and enzymes that control biological processes such as ordered cell migration and adhesion, wound healing, and tissue regeneration. It is composed of the network-forming type IV collagen, laminins, specific PGs containing mainly heparan sulfate (HSPG), and nonscaffolding collagens such as the perisinusoidal collagen type XVIII mainly produced by hepatocytes [28] or collagen type XV located in the portal tract. Type IV collagen is produced by endothelial cells and forms a 3D network instead of fibrils, ideally suited for the incorporation of laminins and proteoglycans. This network forms a low-density matrix along the sinusoids, bile ducts, and vessels of the portal tract. This helps maintain the differentiated and polarized functions of the cells attached to it, notably hepatocytes and cholangiocytes. Type IV collagen can be degraded by matrix metalloproteases to give rise to subdomains with signaling capability, known as matrikines or matricryptins such as tumstatin [29]. Other components of the hepatic ECM comprise GAGs and hyaluronic acid or hyaluronan (HA), a nonsulfated GAG not attached to a core protein.

ECM functions are schematically represented in Figure 1 and can be roughly divided into physical and biochemical properties. In terms of physical properties, the hepatic ECM plays a role in the anchorage of liver cells to confer cohesion to the epithelium (to establish and maintain cell polarity). Hepatic ECM is also a physical barrier to cell migration or conversely can direct this migration through the organization of collagen fibrils in bundles. Liver cells are capable of reacting to biomechanical properties of the hepatic ECM through mechanosensing/-transduction machinery involving the focal adhesion complex and the actin cytoskeleton and ensuring a continuum for signals to propagate from the ECM to the nuclear chromatin. In particular, matrix stiffness and fibrillar architecture can be sensed, generating signals translated into changes in cell shape or behavior [30].

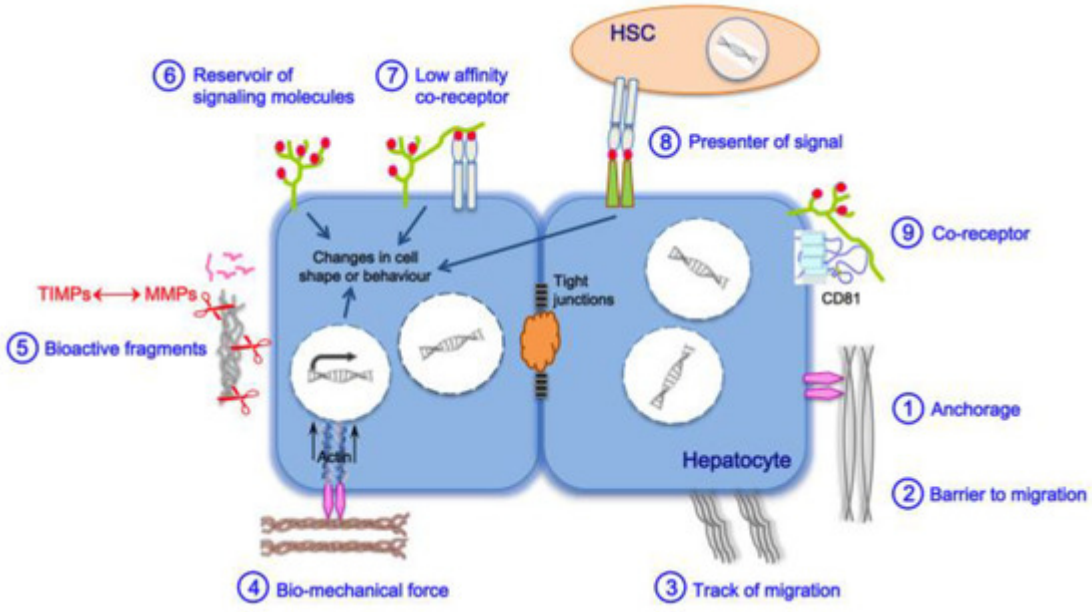

Figure 1. Mechanisms of ECM functions. Biological functions of the ECM are related to its biochemical and biomechanical properties. ① Anchorage to the basement membrane is essential for various processes, such as maintenance of polarity, cell proliferation, and differentiation. ②③ The ECM may also serve to block or guide cell migration. ④ Cells are able to sense the biomechanical properties of the ECM (e.g., stiffness), and change their shape or behavior through mechanotransduction pathways: tensional forces, focused within focal adhesion structures, induce clustering of integrin receptors, which causes recruitment of signaling proteins such as talin, vimentin, paxillin, tensin in direct connection with actin cytoskeletal filaments and microtubules. Several kinases also concentrated at the focal adhesion transfer stimuli from the ECM to intracellular signaling cascades; all these events will ultimately contribute to genome transcription and protein translation. ⑤ The ECM directs signals to the cell through bioactive fragments after their processing by proteases such as MMPs, regulated by TIMPs. ⑥ The ECM acts as a reservoir of signaling molecules by binding and by locally concentrating growth factors, cytokines, and hormones. Some ECM components such as HSPGs can selectively bind to different growth factors and function as low-affinity coreceptors ⑦ or as presenters of signals between hepatocytes and HSCs ⑧, thereby playing a major role in cell–cell communication. ⑨ We demonstrated that the HSPG syndecan-1 and the tetraspanin CD81 interact together; this interaction tightly links the ECM, the tetraspanin web, and likely the cytoskeleton and could have functional consequences on both cell behavior and ECM remodeling. Syndecan-1/CD81 form a coreceptor complex for HCV entry [31].

Biochemical properties of the hepatic ECM include: (i) its ability to capture and bind to growth factors, cytokines, and chemokines and locally concentrate them at the cell surface, thereby acting as a reservoir of signaling molecules. ECM proteoglycans such as heparan sulfate proteoglycans (HSPGs) of the hepatocyte membrane can also bind molecules and function as low-affinity coreceptors or as signal presenters for another cell type present in the space of Disse, with an important role in intercellular communication; (ii) its capacity to send signals to cells, triggered by bioactive fragments of its protein components such as matrikines and matricryptins [29][32], after their processing by MMPs; these processes are regulated by a finely tuned balance between MMPs and TIMPs.

A tight intricacy also exists between the hepatic ECM and cells residing within, with reciprocal interactions contributing to liver homeostasis. Therefore, if any of the physical and biochemical properties of the hepatic ECM are altered, abnormal behavior of cells of the connective tissue will occur, leading with time to the disruption of liver homeostasis and to functional failure observed in fibrosis and cirrhosis.

3. Liver Fibrosis and Cirrhosis

3.1. General Pan-Etiology Features

The main causes of fibrosis are infections with HCV or HBV, alcohol abuse, and nonalcoholic fatty liver disease (NAFLD). Fibrosis is a reversible exuberant wound-healing and scarring process in which excessive connective tissue builds up in the organ (reviewed in [17] for the liver). This dynamic phenomenon is triggered by a chronic liver injury (Figure 2), which causes an imbalance between excessive ECM production (fibrogenesis) and deficient degradation (fibrolysis), during which several cell types are recruited onsite to help “seal off” the injury [33]. Mature scar ECM, composed of cross-linked collagens and elastin, is more resistant to MMPs, and fibrils sequestered in deeper portions of scar become inaccessible to these enzymes [34]. Whatever the etiology, this injury is linked to impaired hepatocyte replicative capabilities, including hepatocyte death, and activates HPCs, i.e., triggers their proliferation and differentiation [35]. This process helps provide new hepatocytes and maintain the organ’s functional integrity and is accompanied by liver inflammation. Cirrhosis is characterized by the disruption of the normal hepatic architecture, with a distortion of the blood flow through the liver: tissue septa form, which connect the incoming vasculature (portal vein and hepatic artery branches) and outgoing vessels (central veins). This may lead to portal hypertension and is accompanied by inflammation, angiogenesis, and hepatic endothelial dysfunction, leading to a global liver dysfunction.

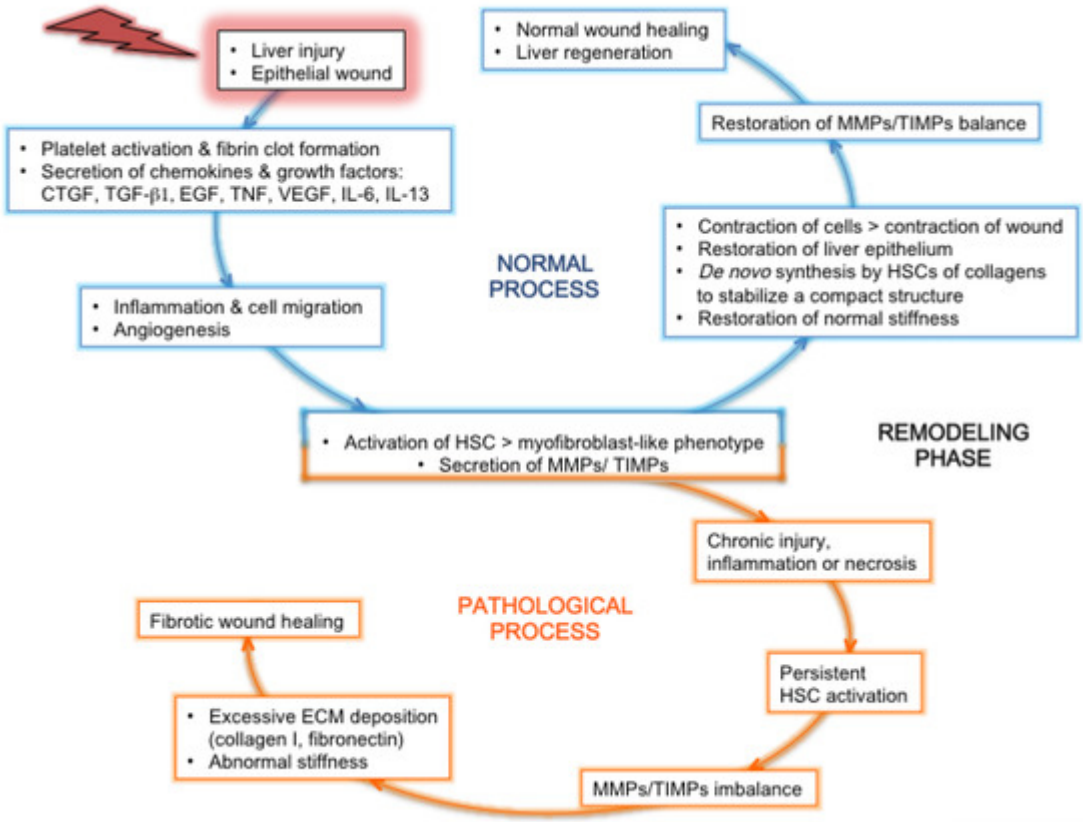

Figure 2. Normal or pathological process after liver injury: tissue regeneration or fibrosis. After the initial event of liver injury, the epithelial wound accompanied by a breach in the endothelium triggers the coagulation cascade, followed by an inflammatory and proliferation phase mediated by the secretion of inflammatory cytokines and growth factors. The profibrotic cytokines IL-13 and TGF-β1 are secreted by activated leukocytes coming from the blood circulation and by sinusoidal cells [36]. Concomitantly, HSCs are activated, thereby adopting a myofibroblast-like phenotype and secreting MMPs and TIMPs. These proteins contribute to ECM remodeling, together with cytokines and chemokines that recruit leukocytes at the site of injury and activate them. In the loop of a normal wound-healing process (**blue**), the inflammatory process gives way to a progressive tissue repair, with the cleaning up of tissue debris and dead cells by leukocytes, the contraction of epithelial cells to restore a normal epithelium, and the de novo synthesis by HSCs of ECM components that organize in order to stabilize a compact structure between and around cells. This helps to restore normal stiffness. In parallel, endothelial cells form new blood vessels. The balance of secretion and activity between MMPs and TIMPs is restored to normal. All these features lead to normal wound healing and liver regeneration. In the loop of a pathological/fibrotic wound-healing process (**orange**), a state of chronic injury and inflammation is maintained, accompanied by tissue necrosis instead of repair. This leads to the persistent activation of HSCs. Thereby, the tight balance between MMPs and TIMPs secretion and activity is disrupted, and overly abundant amounts of ECM components produced by activated HSCs are deposited in the interstitial tissue, which becomes scar tissue with abnormal stiffness. Within this stiffer tissue, the migration of cells and chemokines that could contribute to healing is greatly impaired. Altogether, these features contribute to a fibrotic wound-healing phenotype, with the formation of a permanent fibrotic scar.

Staging of fibrosis is based on liver biopsy and/or noninvasive methods measuring liver stiffness (transient elastography). One of the most commonly used tools to evaluate the severity of chronic liver disease is the METAVIR score, delineating four stages: F0, normal liver; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis. Liver cirrhosis, the most advanced stage, is defined as F4 [37]. Serum biomarkers may be further analyzed as indicators of a higher risk of fibrosis. These markers can be classified as indirect (a combination of routine liver biochemistry and general features) or direct (a reflection of liver extracellular matrix turnover and accumulation). Direct markers include soluble components of the ECM (HA, tissue inhibitor of matrix metalloproteinase-1, collagen byproducts). Liver inflammation related to fibrosis also involves liver-resident macrophages and peripheral monocytes. From that, the frequency of CD14+ monocytes was found significantly higher in HCV fibrotic patients than in healthy individuals and positively correlated with liver fibrosis. Serum levels of CD163, a marker of liver macrophage subpopulation, also correlated with HCV-related liver fibrosis and was proposed as a novel marker for assessing the degree of liver fibrosis in HCV-infected patients [38].

As initial stages of fibrosis are asymptomatic, the diagnosis could be delayed, with delayed implementation of therapy. Indeed, a successful resolution of fibrosis largely depends upon the stage and extent of scarred tissue. Treatments involve correcting the underlying condition when possible, e.g., eliminating excess alcohol consumption, changing to a healthier lifestyle in NAFLD patients, and administering appropriate antiviral therapies to patients with viral hepatitis. This is also valid in the cirrhotic range of fibrosis; de facto, as long as liver functions are maintained, cirrhosis is no longer termed as end-stage disease but as advanced liver disease. However, its therapeutic resolution is more difficult to obtain than that of earlier stages of fibrosis [37], and cirrhotic patients run a 1–7% yearly risk to develop HCC [33].

3.2. Features Linked to HCV Pathogenesis

HCV leaves the circulation through the fenestrae of the sinusoid capillaries and crosses the space of Disse. HCV infection of hepatocytes occurs after recognition at the cell plasma membrane of a quartet of receptors necessary and sufficient for viral entry: the tetraspanin CD81, the scavenger receptor SR-BI, and the components of tight junctions claudin-1 and occludin [39]. Recently, we identified the heparan sulfate proteoglycan (HSPG) syndecan-1 as a cofactor of CD81 for HCV entry [31]; both molecules form a complex linking the ECM to the cytoskeleton [40] and integrins, receptors of ECM components [41]. This emphasizes the subtle connection that occurs early between HCV infection, hepatic ECM, and key components of the intracellular machinery that could act as sensors of ECM physical properties (stiffness/tension; Figure 1).

Persistent HCV infection of hepatocytes induces the activation of the focal adhesion kinase, leading to increased expression of paxillin and delocalization of α-actinin [42], forming the focal adhesion complex [43]. This might translate into modifications of cell adhesion and migration properties and trigger cytoskeletal reorganizations transduced into signals transiting to the nucleus through mechanotransduction machinery (Figure 1). HCV-mediated liver fibrogenesis appears at portal and hepatocellular sites, with ECM deposition around sinusoids in the vicinity of the portal vein as well. This is in contrast with perivenular and perihepatocellular fibrosis, with ECM deposition in the space of Disse, observed in NAFLD- or alcohol-related fibrosis [44][45]. Other specific clinical signs

of HCV-related fibrosis include the clustering of mononuclear cells at the hepatic lobules and the presence of prominent aggregates of lymphocytes in periportal zones [46][47] as indications of a major inflammatory activity not observed in alcohol-related fibrosis [46], also reported in the recently developed rat model of the hepatitis C-like virus [48]. Such clusters of liver-resident macrophages play a key role in liver inflammation through the secretion of proinflammatory cytokines and chemokines [49]. Additional discriminating features include prominent steatosis in HCV-infected hepatocytes [48], as a result of HCV-mediated metabolic reprogramming and necroinflammation, more commonly observed in chronic hepatitis C than B [50] but less than in alcohol-related liver disease [44]. Bile duct damage is also more observed in chronic hepatitis C than B [51]. Bile ductular reactions originate from cholangiocytes or hepatocytes and accompany cholestatic liver diseases such as cholangitis, as well as parenchymal liver cell diseases induced by alcohol and HCV or HBV infections. These reactions are often linked to fibrosis and portal inflammation in chronic liver diseases. During fibrosis, bipotent HPCs produce an excess of fibrogenic mediators, such as transforming growth factors (TGFs) TGF-β1 and -β2, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and sonic hedgehog, supporting HSC proliferation and activation [33]. Interestingly, transcriptomic analyses of HPCs from patients with advanced fibrosis/cirrhosis linked to cholangitis or chronic hepatitis C revealed patterns of gene expression differing in disease etiology [47]. Progenitors from cholangitis patients showed enrichment in morphogenesis and cytoskeleton organization markers, whereas cells from hepatitis C patients displayed an increase in metabolism/hepatocyte markers and networks enriched for cell movement and receptor activity. Ductular reactions in HCV-mediated liver disease were also associated with intense vascular remodeling not observed in cholangitis. Chronic hepatitis C causes major changes in the inflammatory cytokine and chemokine milieu, susceptible to be translated into specific disease manifestations [52]. This agrees with the fact that HCV-associated progenitors and their niche display an increase in invasion- and metastasis-related markers, such as PDGF-α [53] and the insulin-like growth factor-2 [54]. This reveals a striking similarity with cancer progression, i.e., invasion into the parenchyma and (neo)angiogenesis. Additionally, PDGF-α is a profibrotic actor, as it activates HSCs, thereby contributing to the biosynthesis, secretion, and deposition of components of the ECM [55].

The hepatocyte nuclear factor HNF4α is a transcriptional regulator of glycogen metabolism, cell junctions, differentiation, and proliferation in liver and intestinal epithelial cells; it is essential for hepatocyte differentiation during embryogenesis. HPCs from cirrhotic HCV-patient biopsies exhibited nuclear foci of HNF4α, whereas the transcriptional factor c-Jun was more expressed in cells from cholangitis patients [47]. This indicates an etiology-dependent activation of specific transcriptional regulators, HPCs being primed or pushed toward a certain cell fate. In the case of HCV-mediated chronic liver disease, HPCs are therefore pushed toward hepatocytes instead of cholangiocytes [47]. On the path to hepatocellular carcinoma, HPCs are on the contrary maintained in their undifferentiated state, and pushed toward stemness (self-renewal and expansion), under the influence of the lectin galectin-3, as well as α-ketoglutarate, a compound derived from glutamate, both secreted by transformed hepatocytes [56]. Galectin-3, like PDGF, is an activator of HSCs; both molecules therefore play a dual role in HCV pathogenesis at early (fibrosis/cirrhosis) and later (oncogenic transformation) stages of liver disease.

Concerning the ECM, which features could be attributed to HCV-mediated liver disease, possibly linked to the expression of viral proteins? During this pathology, a profibrotic phenotype is acquired, with increased expression

and release in the connective tissue of collagens I and IV [55][57][58], elastin [59], proteoglycans such as fibromodulin [60] or lumican [59], and HA [42]. These overly expressed components, together with dysfunctions of enzymes involved in their metabolism, contribute to alterations in the properties of the hepatic ECM during chronic infection. A spectrum of expression of these and other ECM constituents, enzymes, and regulators of the ECM will therefore be analyzed in the following, in connection with the expression of HCV proteins when identified. Reported connections between HCV proteins and elements of the ECM or cytokines are summarized in Table 2. Correlations between ECM proteins or cytokines expression and METAVIR liver disease/HCC stages in chronically HCV-infected patients are reported in Table 3.

Table 2. Proteins of HCV reported being related to proteins of the ECM or cytokines. *, Direct interaction with the indicated HCV protein; ∞, modulation of expression; ◊, modulation of signaling.

HCV Proteins	ECM Proteins or Cytokines
Capsid core	LOX ∞ [61]
	Procollagen I ∞ [62]
	Collagen I ∞ [61]
	MMP-2 ∞ [58]
	MMP-9 ∞ [63]
	COX-2 ∞ [63]
	Syndecan-1 * [31]
	Thrombospondin-1 ∞ [61]
	Osteopontin * [64][65]
	CTGF ∞ [58]
	TGF-β1 ◊ [58][61][62][66]

HCV Proteins	ECM Proteins or Cytokines
	TGF-β2 ◊ [67]
	Endoglin ∞ [68]
Envelope glycoproteins E1 and/or E2	Glypican-3 * [69]
	TGF-β1 ◊ [66]
Cysteine autoprotease NS2	MICA ∞ [70]
	TGF-β2 ◊ [67]
Serine protease and helicase NS3	Procollagen I ∞ [62]
	MMP-9 ∞ [71]
	COX-2 ∞ [71]
	Thrombospondin-1 [72]
	Osteopontin * [64]
	TGF-β1 ◊ [62][72]
	TGF-β type I receptor * [73]
NS3 with its cofactor NS4A	MMP-9 ∞ [71]
	COX-2 ∞ [71]

HCV Proteins	ECM Proteins or Cytokines
	MICA ∞ [74]
	TGF-β ◊ [72][75]
NS4B	MMP-2 ∞ [76]
NS5A	MMP-2 ∞ [63]
	MMP-9 ∞ [63]
	COX-2 ∞ [63]
	Thrombospondin-1 ∞ [72]
	Osteopontin * [64]
	TGF-β1 ◊ [72][77][78]
RNA-dependent RNA polymerase NS5B	Osteopontin * [64]
	MICA ∞ [70]
	TGF-β ◊ [45][59][60][79][80][81][82][83]

AVIR liver

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Collagen XII	[59][84]
Collagen XIV	[59][84]
Collagen XVI	[59]

ECM Proteins/Cytokine	F0/F1	F2	F3	F4	HCC	References
Collagen XVIII						[59]
PIIINP	F1					[85][86][87]
MMP-2, -7, -9	F1					[63][82][88][89][90]
TIMP-1						[82][86][88][91][92]
ADAM-TS1						[93]
ADAM-TS2						[94]
Xylosyltransferase-2	F1					[95][96]
Glypican-3						[97][98][99]
Hyaluronic acid						[87][100][101]
Decorin	F1					[92]
Biglycan						[59]
Fibromodulin						[60]
Lumican						[59][81][84][102]
Versican	F1					[93][103]

ECM Proteins/Cytokine	F0/F1	F2	F3	F4	HCC	References
Tenascin-C						[104][105]
Osteopontin	F1					[82][106][107]
Fibronectin						[103][108]
Fibronectin isoforms						[108]
Elastin						[59][83][84][102]
MFAP-4 †	F1					[84][109][110]
Fibulin-5						[84]
TGF-β1 (protein, mRNA)						[59][100][103][111][112]
TGF-β1 (serum levels)	F1					[87][113]
TGF-β2	F1			F0		[67]
Endoglin (protein, serum levels)						[100]
Endoglin (mRNA) §						[68]

[a] Color codes: green, upregulation; dark green: higher upregulation; blue, downregulation; dark blue: higher downregulation; grey, no change; magenta, no correlation with liver fibrosis stage. † MFAP-4, microfibrillar-associated protein-4 (associated with elastin fibers). § Endoglin mRNA was found upregulated in chronically HCV-infected patients compared to noninfected patients but not correlating with liver fibrosis stage.

4. Are HSCs Direct Targets of HCV Infection?

Studies addressing the capability of HCV to directly interact with HSCs, that could contribute to a direct fibrogenic effect, have reported conflicting data. Florimond and coworkers showed that human liver myofibroblasts, isolated from liver specimens, and the immortalized HSC cell line LX-2 [114] were not infectable by HCV due to the lack of receptors essential to its recognition and internalization; they also reported that these cells could not support HCV replication [115]. Conversely, Aoudjehane and coworkers showed that human liver myofibroblasts possessed all key receptors necessary and sufficient for HCV entry and supported HCV infection [116]. Infection was followed by overexpression of α-SMA and collagens I and IV. These conflicting data might result from the use of different cell lines and of human liver myofibroblasts infected at different passages. Interestingly, activated HSCs expressed mRNA of all receptors required for productive HCV infection, and incubation of these cells with soluble HCV core or protease NS3 led to the activation of oxidative stress and the stimulation of NF-κB-dependent gene expression pathways [62]. Core preferentially activated pathways involved in cell proliferation, while NS3 acted as a trigger for proinflammatory pathways. The expression of core or NS3 into HSCs led to the increased production of α-SMA, procollagen-I, and TGF-β1 [62]. Although these data did not demonstrate that HSCs could support HCV replication, they suggested that they were potential targets of HCV infection. A recent concept emerged that exosomes secreted by HCV-infected hepatocytes might be “agents” of communication between these cells and HSCs. Indeed, HSCs were found able to internalize exosomes from infected hepatocytes, which led to the upregulation of profibrogenic components (α-SMA, collagens I and III, TIMP-1, MMP-2, CTGF, and TGF-β1) [117]. This upregulation was triggered by the micro-RNA miR-19a, shuttled between infected hepatocytes and HSCs by exosomes. MiR-19a targeted the SOCS3/STAT3 axis, ultimately leading to the engagement and activation of the profibrotic TGF-β1 signaling pathway. This study therefore unraveled a direct mechanism of HSC activation by HCV-infected hepatocytes, although not based upon the infection of HSCs [117].

However, additional studies are needed to fully settle this question, and one must keep in mind that human liver myofibroblasts correspond to activated HSCs, which leaves open the question of HCV infection of quiescent HSCs [118]. Nevertheless, the possibility that (quiescent or activated) HSCs might be directly modulated by the virus during liver invasion enlarges our mechanistic perspectives on the progression of liver disease during chronic infection.

5. Fibrosis Reversal in the Era of DAAs in HCV-Induced Liver Fibrosis

The main angle of attack of hepatitis C-related liver fibrosis is to reduce or eradicate the primary disease, i.e., curing viral infection. With high SVR rates achieved, most patients treated with DAAs have seen their clinical symptoms regress, in particular fibrosis. The recent development of transient elastography has placed the measured liver stiffness as an accurate surrogate index of liver fibrosis, in particular to evaluate the efficacy of DAA treatment [119]. However, such treatment does not fully restore the altered cytokine and chemokine milieu [12], and patients at advanced stages of disease may remain at risk of liver complications. Combining DAAs with other antifibrotic strategies may be desirable, such as therapies aiming at the stimulation of matrix degradation, in

particular the inhibition of LOXL2. The safety, tolerability, and potential efficacy of the anti-LOXL2 monoclonal antibody simtuzumab have been recently assessed in a study of three cohorts of patients: chronically infected by HCV, infected by the human immunodeficiency virus (HIV), or coinfected by HCV and HIV (ClinicalTrials.gov Identifier: NCT01707472 and [120]). Although the treatment was well tolerated, no clinical benefit was observed, with no significant changes in fibrosis score before and after therapy. This might be due to the poor accessibility of simtuzumab to the site of fibrosis, linked to the collagenous consistency of the connective tissue. Other strategies under examination in animals might raise hopes of novel therapies aimed at fibrosis regression, in addition to SVR in chronic hepatitis C patients (reviewed in [17]). Some strategies target intracellular signaling to restore it to normal, such as inhibitors of tyrosine kinase receptors, which are signal transducers of several cytokines. Others aim at inhibiting fibrogenesis by interfering with its main actors, TGF-β1 and CTGF; inhibitors of the TGF-β1 pathway could either block circulating cytokine, antagonize its receptors, and/or block its activation at the cell surface. The monoclonal antibody against CTGF, FG-3019, is currently under clinical investigation in lung fibrosis for safety and tolerability (ClinicalTrials.gov Identifier: NCT01890265) and might be applied to liver fibrosis. Other strategies consist of increasing the apoptosis of activated HSCs through the inhibition of antiapoptotic proteins or transcription factors such as NF-kB. However, although several strategies have been tested in clinical trials lately, their antifibrotic effects have been limited or absent. Thus, to date, no approved therapy exists for liver fibrosis [121].

6. Conclusions and Perspectives

In the era of DAAs, which raises hopes of eradicating HCV, HCV infection remains a leading cause of hepatic failure due to advanced liver disease and HCC because curing the infection does not fully restore liver homeostasis. Furthermore, DAA treatment alone may not be sufficient for a complete cure of fibrosis, as several factors other than the virus contribute to liver deterioration. Lastly, patients under antiviral therapy variably respond to the regression of fibrosis. The mechanism of HCV-induced liver disease is a multifaceted process, as various host genes are altered, and host cells respond to infection/viral components by mobilizing or producing enzymes, growth factors, and chemokines, which activate quiescent HSCs. HCV chronic infection leads to a deep remodeling of the entire liver ECM architecture through direct interactions between viral, ECM, and cellular proteins and indirect effects (e.g., promotion of oxidative and ER stress, inflammation, and stemness). HCV-induced overexpression of TGF-β, the most potent profibrogenic cytokine, contributes to HCV replication and to the activation of HSCs, the promotion of their survival, and the inhibition of HSC apoptosis, mechanisms by which liver disease progresses. Consequently, several general mechanisms involved in liver fibrosis/cirrhosis development contribute to tumorigenesis. TGF-β signaling facilitates HCV replication in hepatocytes and could promote the survival of precancerous cells; furthermore, HCV replicates at higher rates in liver cancer stem cells.

Thus, efforts toward a deeper comprehension of host/virus/ECM interactions and of the underlying mechanisms by which hepatic dysfunctions emerge, spread, and persist after HCV infection are therefore still needed in order to develop therapies that cure liver disease in addition to curing infection.

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