Published in final edited form as:

Semin Liver Dis. 2020 August ; 40(3): 307–320. doi:10.1055/s-0040-1708876.

Hepatic Stellate Cell–Macrophage Crosstalk in Liver Fibrosis and Carcinogenesis

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Abstract

Chronic liver injury due to viral hepatitis, alcohol abuse, and metabolic disorders is a worldwide health concern. Insufficient treatment of chronic liver injury leads to fibrosis, causing liver dysfunction and carcinogenesis. Most cases of hepatocellular carcinoma (HCC) develop in the fibrotic liver. Pathological features of liver fibrosis include extracellular matrix (ECM) accumulation, mesenchymal cell activation, immune deregulation, and angiogenesis, all of which contribute to the precancerous environment, supporting tumor development. Among liver cells, hepatic stellate cells (HSCs) and macrophages play critical roles in fibrosis and HCC. These two cell types interplay and remodel the ECM and immune microenvironment in the fibrotic liver. Once HCC develops, HCC-derived factors influence HSCs and macrophages to switch to protumorigenic cell populations, cancer-associated fibroblasts and tumor-associated macrophages, respectively. This review aims to summarize currently available data on the roles of HSCs and macrophages in liver fibrosis and HCC, with a focus on their interaction.

Keywords

cancer-associated fibroblasts; tumor-associated macrophages; extracellular matrix; hepatocellular carcinoma; cirrhosis

Chronic liver injury is caused by various etiologies, including chronic viral infection (hepatitis B and C viral infections), alcohol abuse, metabolic disorders (nonalcoholic fatty liver disease [NAFLD]), cholestasis, autoimmune hepatitis, parasitic infection, hepatotoxin exposure, hemochromatosis, and Wilson's disease. Despite their etiologies, insufficient treatment of underlying liver disease leads to progressive liver fibrosis. Patients with cirrhosis, an advanced form of liver fibrosis, have poor prognoses due to complications including ascites, portal hypertension, and liver failure. Liver fibrosis is the 11th leading cause of death worldwide, and, to date, 1.16 million people die annually due to cirrhosis in the world.[1]

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Conflicts of Interest

None.

Liver cancer develops in the chronically injured liver. Primary liver cancers are the second leading cause of cancer-related death and the fifth most common cancer worldwide.[2] Approximately 800,000 patients die with primary liver cancer each year globally. Hepatocellular carcinoma (HCC) comprises 80 to 90% of primary liver cancer cases,[3] and cholangiocarcinoma accounts for 6 to 15%. The risk of HCC differs among etiologies of underlying liver diseases: the risk is higher with chronic hepatitis B and C viral infections and lower with alcoholic liver disease or NAFLD.[4] Although the major risk factors for developing HCC are hepatitis B and C viral infections, chronic alcohol consumption and NAFLD are emerging as additional risk factors, especially in Western counties. Nonalcoholic steatohepatitis (NASH), a progressive form of NAFLD, is characterized by excessive lipid storage in hepatocytes, hepatocyte ballooning, liver inflammation, and fibrosis. NASH is a form of NAFLD with a higher risk of HCC development than NAFLD without NASH. Of note, all these etiologies for HCC share common underlying liver conditions such as chronic liver inflammation and fibrosis; 80 to 90% of HCC cases develop in fibrotic or cirrhotic livers.[5] Accordingly, fibrosis and cirrhosis are high-risk factors for HCC development. In patients with cirrhosis, 5 to 30% developed HCC within 5 years.[5] Hepatitis B surface antigen-positive patients with a high serological fibrosis index (FIB-4) had up to a 15-fold increased risk of future HCC incidence,[6] and elevated liver stiffness measured by noninvasive approaches was positively correlated with HCC development in patients with hepatitis B and C viral infections.[7–9] Also, underlying liver fibrosis is associated with a high recurrence rate of HCC after curative therapy. Thus, the fibrotic liver microenvironment is predisposed to developing HCC.[10]

Although it is obvious that fibrosis is strongly associated with HCC development, it is still unclear whether fibrosis is the cause and promoter of HCC. Fibrosis may result as a consequence of chronic liver disease and thus may be a bystander of chronic liver disease. However, collagens, major extracellular matrix (ECM) components that accumulate in liver fibrosis, might promote HCC development through collagen-specific receptors such as integrins. Moreover, fibrosis-related factors, including inflammatory cytokines, regenerative growth factors, and genotoxic factors, could be important functional contributors to HCC development.

Pathological elements that contribute to fibrosis progression are also commonly observed in the HCC microenvironment, and many are associated with HCC development, suggesting that liver fibrosis is a premalignant condition associated with a high risk of HCC development. Mesenchymal cells and macrophages, two key players in fibrosis progression, also play critical roles in tumor development as cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs), respectively. The phenotype and function of CAFs and TAMs associated with HCC lesions could be affected by HCC-derived intrinsic factors and might differ from those in fibrosis without tumors. Accumulating evidence for the mechanistic role of liver macrophages and HSCs in fibrosis could provide a foundation to better understand the roles of CAFs and TAMs in HCC development. In this review, we summarize currently available evidence of the roles of HSCs and liver macrophages in fibrosis and HCC, and we discuss how their interaction modulates fibrosis and HCC development.

Molecular Mechanisms of Liver Fibrosis and HCC Development

Liver Fibrosis

The liver consists of various cell types including hepatocytes, cholangiocytes, liver sinusoidal endothelial cells (LSECs), mesenchymal cells (e.g., HSCs), and immune cells (e.g., macrophages, lymphocytes), all of which cooperatively contribute to liver function and maintain liver homeostasis (Fig. 1). When the liver is chronically injured, fibrosis develops in the interstitial space of the liver, and the liver architecture is distorted, leading to a disruption of cellular homeostasis and dysfunction of the liver. In the last decades, experimental studies using animal models of liver fibrosis have unveiled many underlying mechanisms of liver fibrosis.[11] Liver fibrosis involves complex interactions of multiple cell types in the liver. Fibrotic responses are triggered by damage to hepatocytes and cholangiocytes. Hepatotoxin, alcohol, and hepatitis B and C virus infections cause hepatocyte damage. Damaged hepatic cells release damage-associated molecular patterns (DAMPs) including nuclear proteins (e.g., HMGB-1), cytokines (e.g., interleukin [IL]-1α, IL-33, S100A8/9), intracellular molecules (e.g., Hsp70), and mitochondrial components (e.g., mitochondrial DNA).[11,12] In chronic liver diseases, such as alcoholic steatohepatitis, increased intestinal permeability causes the translocation of intestinal bacterial products (e.g., lipopolysaccharides [LPS]) to the liver through the portal vein as pathogen-associated molecular patterns (PAMPs).[13] Although Kupffer cells, the liver-resident macrophages, are the primary hepatic cells to respond to LPS through toll-like receptor 4 (TLR4), HSCs are also activated by LPSs through TLR4. Both Kupffer cells and activated HSCs contribute to fibrosis progression in alcohol-induced fibrosis through TLR4.[14] These DAMPs and PAMPs cooperatively trigger an innate immune response that involves inflammation in the liver. Once activated, HSCs begin to produce fibrous collagens and ECM remodeling factors such as matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). When injuries continue, HSCs are persistently activated, leading to excessive production and accumulation of ECM (i.e., fibrosis).[11] Activated HSCs also secrete chemokines to attract immune cells,[15] including neutrophils, macrophages, natural killer (NK) cells, NKT cells, innate lymphoid cells, B cells, and T cells.[12,16] These immune cells further activate HSCs through cytokine production or direct interactions. Among immune cells, macrophages are the key regulators of fibrosis progression.[13,17,18] Macrophages regulate HSC functions by producing profibrogenic cytokines. Activated HSCs then produce large amounts of ECM. Macrophages also contribute to the remodeling of deposited ECM through MMP production. Compositional ECM changes, in turn, affect the functions of the surrounding cells in the fibrotic niche by promoting differentiation including phenotypic and functional switches of the immune cells as well as HSCs.

HCC Develops in Underlying Liver Fibrosis

Most HCC cases develop in the chronically injured liver. Pathological features of chronic liver injury include fibrosis (increased and remodeled ECM), inflammation (accumulated immune cells, cytokines), reactive oxygen species (ROS), and compensatory hepatocyte regeneration, all of which contribute to the development of a liver microenvironment favorable toward tumor growth. Therefore, liver fibrosis can be considered a pre- and promalignant environment for HCC.

Previous studies using animal models have proposed the molecular mechanisms by which HCC is promoted by profibrogenic factors including cytokines, growth factors, chemokines, and angiogenic factors expressed in fibrotic livers.[12] Platelet-derived growth factor (PDGF)-C, a fibrogenic cytokine, promotes hepatocarcinogenesis,[19] supporting the hypothesis that underlying liver cirrhosis has carcinogenic potential. Increased angiogenesis in the fibrotic liver can also promote HCC development.[20–22] Suppressing angiogenesis by inhibiting vascular endothelial cell growth factor (VEGF) can be a therapeutic strategy for HCC.[23,24]

Abundant ROS accumulation, as observed in the chronically injured liver,[25,26] is an important HCC-promoting factor. Mechanistically, ROS induce DNA damage and genomic instability in hepatocytes of hepatitis B virus-infected and alcohol-damaged livers.[27] Also, ROS inhibit $CD4^+$ T cell mediated tumor surveillance, assisting HCC progression in NASH-associated HCC.[28] Indeed, ROS inhibition prevented HCC development in animal models.[29,30]

The immune microenvironment also plays a critical role in HCC development.[31,32] Decreased antitumor T-cell function is commonly observed in various cancers including HCC. Impaired antitumor T-cell function correlates with the upregulation of inhibitory receptors—such as programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)—that is associated with a poor prognosis for HCC patients.[33,34] Inhibition of immune checkpoint point molecules, including PD-1, PD ligand (PD-L)-1 (PD-L1), and CTLA-4, has been shown to dramatically improve treatment of selected cancers through restoring antitumor T-cell function.[35,36] However, the treatment of HCC with immune checkpoint inhibitors has shown only minimal or marginal effects,[37] suggesting that an antitumor T-cell effect would not be enhanced by the currently available immune checkpoint inhibitors in most HCC patients. Instead, a separate immunosuppressive mechanism could be associated with HCC progression. The HCC immune microenvironment may be unique due to the underlying pathological liver conditions including liver fibrosis. HCC-derived factors may also influence the cells surrounding HCC to switch their phenotypes, creating a unique HCC tumor microenvironment (TME). TMEs consist of endothelial cells, stromal cells such as CAFs, and immune cells including TAMs. These components support tumor growth and metastasis in the TME through unique mechanisms (Fig. 2, discussed in the following section). In addition, it is suggested that HSC and macrophage activities synergistically promote HCC development. The HSC–macrophage interplay could contribute to the induction of myeloid-derived suppressor cells (MDSCs) that suppress immune cell tumor surveillance and promote pre- and promalignant microenvironments predisposed to HCC.[38,39] ECM stiffness determined by HSCs also affects proliferation and chemotherapeutic response of HCC cells.[40]

In summary, liver fibrosis precedes HCC development, and both fibrosis and HCC share common exacerbating factors. Immune tolerance and fibrosis are unique features in HCC development, and HSCs and macrophages are the key cell types that regulate both fibrosis and HCC through various mechanisms. In the following sections, the mechanistic roles of HSCs and macrophages in fibrosis and HCC are discussed. A subsequent section further discusses the interactive roles of HSCs and macrophages in fibrosis and HCC.

The Role of Hepatic Stellate Cells in Liver Fibrosis and Cancer

Hepatic Stellate Cells Are Key Player in Liver Fibrosis

HSCs, hepatic mesenchymal cells, reside in the perisinusoidal space of Disse between the fenestrated liver endothelium and epithelial hepatocytes (Fig. 1). Quiescent HSCs store 80% of the body's total vitamin A (retinol) as retinol ester within cytoplasmic lipid droplets and regulate its transport and storage.[41] HSCs are activated by various stimuli (summarized in Table 1) that include profibrogenic cytokines transforming growth factor-β (TGF-β)[42] and PDGF,[43–48] inflammatory cytokines (e.g., tumor necrosis factor-α [TNF-α], IL-1β), chemokines (chemokine ligand [CCL] 2, CCL5), DAMPs, and PAMPs.[11] During the activation process, HSCs lose the storage of vitamin A containing lipid droplets and express α-smooth muscle actin (α-SMA) and ECM components including collagen, fibronectin, and hyaluronan (HA). In addition to the production of ECM, HSCs can also regulate ECM turnover in the space of Disse by producing degrading enzymes (MMPs and TIMPs).[49] In activated HSCs, TIMP1 is upregulated and suppresses MMP activity to prevent ECM degradation, thereby promoting ECM accumulation and leading to liver fibrosis.[11] For HSC activation, TGF-β plays a critical role by activating the transcription factor SMAD2/3/4 complex, transcriptionally upregulating α-SMA, collagen, and HA synthase 2 (HAS2), the enzyme that synthesizes HA. TLR4 signaling also plays a crucial role in enhancing TGF-β signaling by downregulating BAMBI (bone morphogenetic protein membrane-bound inhibitor), an endogenous inhibitor for TGF-β receptor type I.[50] Furthermore, both TGF-β and TLR4 signaling suppress the expression of miR-29 that negatively regulates type I, III, IV collagen, thereby upregulating collagen expression.[51]

Interestingly, deposited ECM can also affect HSC phenotypes. HSC express ECM receptors for collagens, discoidin domain receptor (DDR), and integrins. Type I collagen, a dominant ECM protein in the fibrotic liver, contributes to HSC activation through the integrin and Yes-associated protein (YAP) pathway.[52–54] DDR also contributes to HSC activation.[55,56] Other ECM components, such as the glycosaminoglycan HA, also play critical roles in fibrosis progression. Activated HSCs overexpress HAS2 that synthesizes HA, and then the overproduced HA promotes HSC activation and fibrosis through CD44/Notch signaling.[57] ECM stiffness also contributes to HSC activation. Compositional changes and crosslinking of collagen fibers by lysyl oxidaselike enzyme (LOXL) contribute to the increased stiffness of fibrosis, which activates HSCs through mechanosensing signaling, including the YAP-activating pathway.[58] Hypervascularity and angiocrine factors secreted from the endothelial cells also promote HSC activation in chronic liver injury.[59]

In addition to ECM production, activated HSCs regulate vascular and immune systems. In liver fibrosis, HSCs promote angiogenesis by producing angiopoietin I.[60] Moreover, activated HSCs increase the expression of α-SMA, which is also associated with HSC's contractile ability and regulates vascular tone through the secretion of vasoconstrictive agents, such as angiotensin I and II, that contribute to portal hypertension.[61] Activated HSCs also have an immunoregulatory role. HSCs promote the infiltration of immune cells to the injured liver by secreting cytokines (e.g., IL-6)[11] and chemokines (e.g., CCL2, CCL5).[62] Activated HSCs can also switch the phenotype of macrophages to support fibrosis.[63]

Furthermore, activated HSCs express PD-L1,[64] which plays a crucial role in regulating T-cell apoptosis.

Although myofibroblasts are the chief producers of collagen and responsible for liver fibrosis progression, the origin of myofibroblasts in the chronically injured liver is still a topic of debate. A lineage-tracing study using lecithin retinol acyltransferase (Lrat) promoter-driven Cre recombinase/reporter mice demonstrated that approximately 85 to 95% of myofibroblasts are derived from Lrat-expressing HSCs in rodent liver fibrosis models of hepatotoxin-induced injury, cholestasis, and NASH.[65] HSCs are considered the main precursors of myofibroblasts in most chronic liver diseases. Other studies suggest that myofibroblasts are also derived from other mesenchymal cell populations, including portal fibroblasts, mesothelial cells, fibrocytes, and mesenchymal stem cells.[66] Portal fibroblasts may play a significant role in fibrosis associated with cholestasis.[67]

The Association of HSCs and CAFs in HCC Development

Cancer is associated with a unique tissue microenvironment, consisting of tumor cells together with nontumor endothelial cells, immune cells, and stromal cells. The fibroblastic type of cells in stromal components surrounding cancer cells is termed CAFs. Although there is no specific unique marker of CAFs, αSMA is used as the cellular marker of CAFs in various cancers, and α-SMA+ myofibroblasts are often observed around HCC. As HCC develops in fibrotic livers, myofibroblasts also accumulate in adjacent nontumor liver tissues, which are seen in the fibrotic liver prior to HCC development. The functional differences between myofibroblasts in adjacent fibrotic liver tissues and HCC are still unknown. The cellular origin of CAFs surrounding HCC is generally considered HSCs, but evidence from a lineage-tracing analysis is still lacking.

Clinical evidence supports an association between CAFs/myofibroblasts and the prognosis of patients with HCC. In patients with curative HCC resection, a high degree of peritumoral myofibroblasts and CAFs is associated with a2.6-fold increased risk of death and a 3.3-fold increased risk of recurrence,[68] and several mechanisms for this association have been proposed (Fig. 2). First, CAFs directly promote tumor growth and survival through the production of cytokines and growth factors including TGF-β, hepatocyte growth factor (HGF), and epiregulin.[69–71] Second, CAFs contribute to the production and remodeling of the ECM surrounding HCC, leading to progression (discussed in the following). Third, CAFs promote angiogenesis by producing angiogenic factors such as VEGF and HGF.[21,72,73] Fourth, CAFs suppress immune surveillance of HCC by inhibiting lymphocyte infiltration to tumors, inducing apoptosis of infiltrating mononuclear cells, promoting the infiltration of immuno suppressive regulatory T cells, and inducing an immunosuppressive phenotype of monocytes, MDSCs, in a cell–cell contact-dependent manner.[74–76]

The CAF induction mechanism is not fully understood in HCC but has been proposed in other cancers (e.g., melanoma and breast cancer). It is suggested that tumor cell derived factors contribute to the induction and recruitment of CAFs. For example, in human melanoma cells, oncogenic BRAF (V600E) signaling perturbs antitumor T-cell responses by modulating CAF phenotype. BRAF in melanoma drives the production of IL-1α and IL-1β, thereby enhancing the CAF capability to suppress melanoma-specific cytotoxic T

lymphocytes, in part through COX-2 secretion and upregulation of PD-L1 and PD-L2.[77] In addition, a unique phenotype of CAFs seems to be induced by intrinsic factors derived from other types of cells in the TME, including macrophages (discussed in a later section). Another study found that in breast cancer, the CAF phenotype is induced by mechanosensing YAP signaling.[78] YAP signaling modulates the CAF feature, resulting in the production of ECM. Increased ECM accumulation further increases stiffness, which again activates YAP signaling, establishing a feed-forward self-reinforced loop in CAFs. Transient ROCK (rho-associated coiled-coil kinase) inhibition causes a long-lasting reversion of YAP signaling, suggesting that disrupting the feed-forward loop of the stiffness-YAP signaling in CAFs could be a potential target for cancer therapy.[78]

Somatic mutations are a landmark feature of cancer and a critical driver of transformation to cancer cells. While stromal cells surrounding tumors are genetically stable compared with rapidly growing tumor cells, previous reports studying breast cancer demonstrated mutations in stromal cells including CAFs.[79,80] It is possible that somatic mutations of CAFs are involved in CAF induction and presumably modulation of the TAM phenotype in HCC. Mutations caused by DNA damage and excessive ROS production may be associated with cellular senescence including HSC senescence. HSC senescence is proposed to contribute to HCC development, although these studies have not defined senescent HSCs as CAFs. One study demonstrated that ablation of a p53-dependent senescence program in HSCs augments liver fibrosis and enhances the transformation of adjacent epithelial cells into HCC. In addition, p53-expressing senescent HSCs release the factors that skew macrophage polarization toward tumor-inhibiting M1 macrophages capable of attacking senescent cells in culture; in contrast, proliferating p53-deficient HSCs secrete factors that stimulate polarization of macrophages into tumor-promoting M2 macrophages and enhance the proliferation of premalignant cells.[81] However, a separate study showed a contradictory result that senescence-associated secretory phenotype (SASP) of HSCs promotes HCC development. In this study, high-fat diet feeding induced an alteration of gut microbiota, thereby increasing the influx of deoxycholic acid, a gut microbial metabolite that causes DNA damage, into the liver.[82] It is suggested that proinflammatory SASP by senescent HSCs is required for HCC initiation, but senescent HSCs can also mediate antitumor effects in established HCC. Further studies are still required to investigate the impact of HSC senescence and SASP in HCC.

The Role of Macrophages in Liver Fibrosis and Cancer

Macrophages also play a critical role in liver homeostasis and disease development. When chronic liver disease develops, the macrophage phenotype dynamically changes depending on the duration and degree of inflammation, fibrosis, and cancer. Macrophages in the chronically injured liver have heterogeneous phenotypes due to their high plasticity in response to local environmental stimuli. The M1–M2 paradigm has been used to classify the functional heterogeneity of macrophages. M1 macrophages represent a proinflammatory and antitumor phenotype, expressing TNF-α, IL-1β, IL-12, CCL2, iNOS (inducible nitric oxide synthase), and ROS. In contrast, M2 macrophages are anti-inflammatory and protumorigenic, expressing arginase-1 and CD206, and may be profibrogenic. In addition, liver macrophages can be divided into at least two different ontogenies, yolk sac-derived and

bone marrow monocyte-derived. Different origins of macrophages could show that distinct functions such as phagocytic capability and cytokine production are different between these two origins. Recent studies shed light on the various functions of liver macrophage subpopulations with different origins in liver homeostasis, inflammation, and fibrosis. In the following sections, currently available evidence is summarized, and we discuss how each subpopulation contributes to the pathogenesis of fibrosis and HCC development.

The Different Ontogeny and Roles of Macrophages in Liver Homeostasis and Inflammation

The liver contains a large number of tissue-resident macrophages known as Kupffer cells. Kupffer cells originate from the macrophage population distributed into liver tissues in the embryonic period and are maintained in the adult liver through their self-renewal capacity without any contribution from adult bone marrow monocytes.[83] Kupffer cells are generally adapted to and tolerogenic to hepatic environmental stimuli, which prevents the immune response to the continuous exposure to gut microbiota derived PAMPs and food-derived toxins in the healthy liver. Thus, Kupffer cells play a central role in the immune tolerance of the liver. The scavenger receptors, complement receptors, and TLRs expressed by Kupffer cells enable them to capture, phagocytose, and internalize circulating pathogens and harmful substances in the blood stream. Hepatic immune tolerance is associated with the production of anti-inflammatory cytokines from Kupffer cells, the downregulation of costimulatory molecules on antigen-presenting cells, and inhibition of the T-cell activity.[84] In contrast, in the injured liver, hepatic immune tolerance is disrupted, and the liver becomes inflammatory.[85] In chronic viral hepatitis, Kupffer cells are activated by viral-derived PAMPs through TLR3 and TLR9. They induce phenotypic changes in antigen-presenting cells, which further induce a robust T-cell response.[86] In alcoholic liver disease, Kupffer cells respond to gut-derived LPS through TLR4. Excessive alcohol consumption disrupts gut epithelial tight junctions, which increases intestinal permeability, translocating gut-derived LPS to the liver.[87,88] In this context, Kupffer cells secrete inflammatory cytokines and chemokines (e.g., IL-6, TNF-α, and CCL2) that recruit inflammatory cells and induce inflammatory reactions.[89] Indeed, Kupffer cell depletion reduces liver inflammation in various experimental models of liver diseases, underscoring that Kupffer cells play the sentinel role in response to various environmental stimuli in the liver. Kupffer cells also play a pivotal role in maintaining metabolic homeostasis in the liver, including iron metabolism through phagocytosis of aged red blood cells, and lipid metabolism.[90,91]

The second type of liver macrophage is the monocyte-derived macrophage population, which highly express CCR2. In the injured liver, monocytes from peripheral blood or bone marrow infiltrate and differentiate into macrophages.[92] The monocyte-derived macrophages contribute to inflammation and the wound healing response through the production of pro- and anti-inflammatory cytokines in response to environmental stimuli. Responses to environmental stimuli may be different among macrophages with different origins. In alcohol or high-fat diet-induced steatohepatitis mouse models, Kupffer cells are barely activated, but monocyte-derived macrophages show an evident inflammatory phenotype in a NOTCH-dependent manner.[93] These findings suggest that the monocyte-derived macrophages do not have tolerogenic activity to environmental stimuli when they infiltrate and are highly susceptible to environmental stimuli.

Taken together, upon liver injury, Kupffer cells and monocyte-derived macrophages have different roles; Kupffer cells have the sentinel function to phagocytose harmful environmental substances and regulate hepatic immune reaction, whereas monocyte-derived macrophages are the cells that produce inflammatory cytokines and contribute to the liver macrophage pool for regulating hepatic inflammation and wound-healing response in a timely manner. However, the diversity of liver macrophage functions depends on their ontogenetic origin, and it is more complicated in liver fibrosis and HCC pathogenesis. Recent studies indicate that infiltrating monocyte-derived macrophages differentiate into the Kupffer cell-like phenotype,[94] as described by their acquisition of phagocytic tissue-resident Kupffer cell functions, upon 60 days of repopulation after liver injury.[95] Although it is still controversial whether the newly repopulated monocyte-derived tissue macrophages are long-lasting or short-lived or whether the susceptibility to environmental stimuli and functional roles are comparable to the original yolk sac-derived Kupffer cells.[94,96] To understand the role of macrophages in chronic liver disease, functional plasticity and origin should be further elucidated.

Liver Macrophages Mediate Liver Fibrosis Progression

Macrophages produce various profibrogenic factors including cytokines and chemokines (Table 1). Among macrophage-derived profibrogenic factors, TGF-β is one of the most potent fibrogenic cytokines, and liver macrophages are the primary source of TGF-β in liver fibrosis.[42] The distinct roles of macrophages between bone marrow origin and resident Kupffer cells in liver fibrosis are being investigated. CCR2, the chemokine receptor for CCL2, is predominantly expressed in bone marrow derived macrophages when compared with resident Kupffer cells. In the mouse NASH study, Kupffer cells are the primary cells to be initially activated, producing CCL2. The Kupffer cell-derived CCL2 then recruits bone marrow derived monocytes that express Ly6C and CCR2 in the liver, promoting NASH. Thus, Kupffer cells are required for inducing an initial inflammation, and subsequently bone marrow derived monocytes play a major role in the progression of NASH and fibrosis.[97] CCL5 produced from liver macrophages and HSCs contributes to the infiltration of liver macrophages and HSCs through CCR1 and CCR5, respectively.[98] Based on these findings, recent studies demonstrated the potential of chemokine receptors and monocyte-derived macrophages as therapeutic targets for NASH fibrosis. In patients with NASH,[99] as well as mouse NASH models,[100,101] treatment with the CCR2/CCR5 dual inhibitor prevented monocyte-derived macrophage recruitment and HSC activation and suppressed fibrosis. These data suggest that the CCR2/CCR5 dual inhibitor has the potential to treat NASH fibrosis. Moreover, in a NASH model using melanocortin-4 receptor-deficient mice fed a Western diet followed by a low-dose carbon tetrachloride injection, the depletion of $CD11c^{+}$ liver macrophages—shown to develop from resident Kupffer cells and strongly associate with NASH features in this study—prevented NASH fibrosis.[102] These studies suggest that both resident Kupffer cells and bone marrow derived infiltrated macrophages are crucial in developing NASH fibrosis.

Another recent study identified a specific fibrogenic macrophage subpopulation. $Ceacam1^{+}Msr1^{+}Ly6C^{-}F4/80^{-}Mac1^{+}$ monocytes found in the high-fat diet-induced fatty liver model contribute tothefibrosis progression.[103] This monocyte subset is derived from

Ly6C^{-}FcεRI^{+} granulocyte/macrophage progenitors and share the granulocyte characteristics, termed *segregated nucleus-containing atypical monocyte* (SatM), based on its unique morphological feature. The study also showed that SatMs contribute to the development of liver fibrosis as well as lung fibrosis, in which CEBPB (CCAAT enhancer binding protein β) plays a role.

A more recent RNA-sequencing study at single-cell levels demonstrated the heterogeneity of liver macrophages in the human fibrotic liver.[104] This study identified TREM2^{+}CD9^{+} macrophages as the scar-associated macrophage (SAM) sub-population, and their subsequent RNA trajectory analysis suggested that this SAM subpopulation is of circulating monocyte origin. These findings suggest that bone marrow derived macrophages show a more fibrogenic phenotype. Effectors of macrophages responsible for activating HSCs and promoting fibrosis include TGF-β and IL-1β.[50,105]

Liver Macrophages Mediate HCC Development

Liver macrophages are the key contributors to HCC initiation, progression, and metastasis. In mouse HCC models, TLRs and MyD88-mediated signaling contribute to liver macrophage activation.[106,107] Liver macrophages then produce IL-6, promoting hepatocarcinogenesis through STAT3 activation.[106] Thus, the proinflammatory liver macrophage phenotype is important for HCC initiation in mice. In contrast, the immunosuppressive liver macrophage phenotype can also create an environment favorable for HCC development. Once HCC develops, a unique macrophage population emerges comprising TAMs. TAMs play an essential role in supporting tumor growth through various mechanisms including inhibition of antitumor T cells, activation of CAFs, and remodeling of ECM. TAMs often counteract the antitumor effect of T cells. T cells are one of the critical players for tumor surveillance and have a potent antitumor effect. However, in the TME, the tumor-associated immunosuppression mechanism inhibits the activity of antitumor T cells.[108,109] In chronic viral infection,[110] antitumor T-cell dysfunction shares many features with T-cell exhaustion, such as high expression of inhibitory receptors (PD-1, CTLA-4, TIM-3, LAG-3, and 2B4), loss of effector functions such as production of interferon-γ, and loss of proliferative capacity.[111] Previous studies demonstrated the mechanistic role of macrophages in the induction of T-cell dysfunction during the development of cancers other than HCC and also suggested that the mechanism is related to different origins of macrophages (tissue-resident macrophage-derived TAMs vs. bone marrow monocyte-derived TAMs).[112] In mouse pancreatic ductal adenocarcinoma, tissue-resident macrophage-derived TAMs are more supportive of tumor growth than monocyte-derived TAMs. While TAMs could suppress CD103^{+} dendritic cells (DCs) through secretion of IL-10, tumor burden was reduced only by the loss of tissue-resident macrophage-derived TAMs but not bone marrow monocyte-derived TAMs. This suggests that tissue-resident macrophage-derived TAMs have an immunosuppressive effect in this model.[113] On the contrary, in breast cancer, the proportion of exhausted T cells is simultaneously increased with that of bone marrow monocyte-derived TAMs. In this study, depletion of monocyte-derived TAMs, but not of tissue-resident macrophages, relieved suppression of cytotoxic T cells.[114] The heterogeneity and the context-dependent functions of TAMs may account for the inconsistency of the roles of TAMs between different studies as well as different cancers. In HCC, it is speculated that

resident Kupffer cell-derived TAMs may contact antitumor T cells in the initial stage of HCC development and contribute to the early T-cell exhaustion because resident Kupffer cells are present in the tumor site before tumor progression. Not only the origin but also the functional plasticity determines the macrophage phenotypes and their roles in tumor progression. The molecular switches that control macrophage phenotypes from immunostimulatory to immunoinhibitory have also been investigated. In cancer, inhibition of BTK (Bruton tyrosine kinase) or PI3Kγ,[115,116] master inducers of immunosuppressive phenotype in macrophages, restore antitumor T-cell function, suggesting the involvement of these pathways in promoting immune tolerance. The cue for the phenotypic switch and the origin of TAMs during HCC development in the fibrotic liver remains to be elucidated.

The HSC–Macrophage Interaction in Fibrogenesis and HCC

The interplay between HSCs and macrophages is critical for liver fibrosis progression. Experimental models have demonstrated the molecular mechanisms by which HSCs and macrophages reciprocally regulate their phenotypes through the production of various cytokines and chemokines to induce fibrogenic phenotypes and modulate the ECM. The increased ECM, in turn, affects the cellular functions through ECM-specific receptors and mechanosensing mechanisms, further progressing fibrosis. Given that fibrosis is a premalignant environment, the interplay between HSCs and macrophages should also be important in HCC development. In addition to ECM remodeling during liver fibrosis progression, CAFs and TAMs cooperatively remodel the ECM surrounding HCC that forms its unique TME. CAFs and TAMs also contribute to the immunosuppressive phenotype in TME immune cells, creating a unique microenvironment around HCC.

Crosstalk of HSCs and Macrophages is Crucial for Liver Fibrosis Development

It has been demonstrated that depletion or blockage of macrophage infiltration reduces HSC activation and fibrosis, suggesting the critical role for macrophage–HSC interplay during fibrosis progression.[50,97,117] Macrophages produce various mediators that activate HSCs. These macrophage-derived profibrogenic mediators include TGF-β, PDGF, oncostatin M (OSM), IL-1β, and TNF-α (Table 1). In addition to HSC activation, liver macrophages are known to support the survival of activated HSCs and thereby promote liver fibrosis, in which IL-1β and TNF-α play a role.[118] This is consistent with the notion that apoptosis of activated HSCs is associated with suppression and resolution of fibrosis.[118] On the other hand, activated HSCs attract monocytes/macrophages through the production of chemokines such as CCL2.[15] Infiltrating monocytes/macrophages can further activate HSCs. In addition to chemokines, HA produced from activated HSCs also attracts liver macrophages and HSCs. Both liver macrophages and HSCs express Jagged-1, a membrane-bound ligand for Notch receptors. Jagged-1 expressed on liver macrophages and HSCs could interact with Notch1 on HSCs, promoting Notch1-mediated HSC activation and fibrosis.[57] These observations support that the bidirectional regulation between macrophages and HSCs is the key mechanism of fibrosis progression.

In contrast to fibrosis progression, infiltrating monocytes/macrophages also contribute to fibrosis regression through the production of antifibrotic mediators such as MMP12 and

MMP13.[119,120] In carbon tetrachloride-induced liver fibrosis, the cessation of carbon tetrachloride administration causes spontaneous resolution of liver fibrosis. In this model, macrophages have opposing roles in fibrosis progression and regression: depletion of macrophages inhibited fibrosis progression, whereas depletion of macrophages after injury cessation suppressed fibrosis resolution, resulting in more fibrosis.[121] While the molecular mechanism by which macrophages switch from profibrogenic to restorative phenotypes has not been fully uncovered, phagocytosis is suggested as a trigger for this macrophage phenotypic switch. Taken together, infiltrating monocytes/macrophages can be both profibrogenic and anti-fibrogenic in a context-dependent manner. An antifibrotic strategy of blocking macrophage infiltration might be therapeutic in the fibrosis progression stage, but it could be pathogenic in theresolution stage. Given the conflicting macrophage functions in fibrosis, it is important to identify how the profibrogenic phenotype of macrophages is induced and maintained in the chronically injured liver. The mechanisms by which activated HSCs regulate the macrophage profibrogenic phenotype have been studied. Coculturing human activated HSCs with peripheral blood mononuclear cell-derived macrophages results in a unique phenotypic change of macrophages that produce IL-6 and TGF-β, suggesting that activated HSCs contribute to macrophage phenotypic change to proinflammatory and profibrogenic. Because p38 inhibition in HSCs abolished this macrophage phenotypic change, p38 activity in HSCs is crucial for the induction of proinflammatory and profibrogenic macrophages.[63] A recent RNA-sequencing analysis at single-cell levels on human cirrhotic liver identified the fibrosis-specific subpopulation of macrophages (SAMs) in the fibrotic livers. SAMs produce epidermalgrowth factor receptor (EGFR) ligand, PDGF-BB, and TNFSF12A (TNF superfamily member 12), contributing to HSCs to be more fibrotic.[104] There are fewer studies investigating the interplay of HSCs with liver-resident Kupffer cells compared with that of HSCs with monocyte-derived infiltrating macrophages. In mouse liver fibrosis, Kupffer cells can activate HSCs and recruit TREM1^{+}BMDM.[122]

ECM Remodeling Plays a Role in Fibrosis and HCC

ECM in the liver not only maintains three-dimensional structure as scaffolding but also acts as signaling molecules through specific receptors and regulates cell fate, differentiation, and functions around ECM (Table 2).[123] In addition, tissue stiffness determined by ECM also modulates cellular functions.[40,124] During fibrosis progression, ECM components are dynamically regulated through production and degradation by HSCs and macrophages, respectively.[123,125] TAMs contribute to remodeling ECM in various cancers through MMP-mediated enzymatic degradation and up-take.[126,127] Thus, both HSCs and macrophages regulate fibrosis and HCC through ECM remodeling.

In the normal liver, the dominant ECM component is type IV collagen, which is found along the sinusoids and contributing to the basement membranes for hepatocytes and LSECs. In contrast, fibrotic livers shift toward the accumulation of fibrillar collagen types I and III.[128] Type I collagen can promote HSC activation through integrin αv/β1 and DDR2.[52–54] In HCC, collagen can promote migratory and invasive phenotypes of tumor cells through DDR2,[129] which is associated with tumor aggressiveness. Interestingly, type I collagen may also regulate macrophage phenotypes. The high-density of type I collagen induces an immunosuppressive phenotype of macrophages, resembling TAMs.[130] Macrophages

cultured with high-density collagen decreased cytotoxic T-cell recruitment and proliferation compared with those cultured with low-density collagen.

Increased ECM components in fibrotic livers also include collagen types IV, VI, VII, X, XIV, XV, XVI, and XVIII, and other noncollagenous glycoproteins such as fibronectin, elastin, decorin, nidogen 1, perlecan, and multiple laminin subunits.[11,131] Laminin supports the expansion and differentiation of liver progenitor cells that are thought to contribute to liver regeneration or adaptation to chronic injury in the liver.[132,133] Furthermore, laminin can contribute to HCC progression.[134] Decorin can act as a suppressor of fibrosis[135] and HCC.[136] In addition, the nonprotein glycosaminoglycan HA also is increasingly deposited in fibrotic livers.[57] Among these ECM components, we recently reported that HA activates HSCs through CD44 and TLR4 in the chronically injured liver.[57] HA can also promote HCC progression through CD44.[137–139] CD44 and TLR4 are expressed in hepatic macrophages and HSCs, suggesting that accumulated HA can affect macrophage as well as HSC functions, and it is likely that HA is a key molecule that regulates both HSCs and macrophages in fibrosis. Increased stiffness in the ECM also promotes fibrosis and HCC development. Cellular mechanosensing of stiffness through integrins leads to YAP activation in HSCs and HCC.[58,78,140] LOXL2 mainly produced from macrophages contributes to increasing stiffness by crosslinking collagen fibers.[141] Thus, the bidirectional regulation of HSCs and ECM plays a role in fibrosis and HCC.

ECM can trap growth factors and cytokines, such as HGF, VEGF, OSM, and TGF-β as ECM-associated proteins. Furthermore, the ECM can function as reservoirs as well as coreceptors. Remodeling of ECM by MMPs causes the release and alteration of these molecules, which may affect fibrosis and HCC progression.[128]

Immunosuppressive TME Is Induced by the Cooperation of HSCs and Macrophages

While the normally functioning immune system has the ability to remove cancerous cells by cytotoxic CD8 T cells, $CD4^+$ Th1 T cells, NK (natural killer) cells, and DCs,[142] cancer cells often evade immune surveillance. This evasion mechanism includes the production of immunosuppressive factors, such as TGF-β, and the accumulation of immunosuppressive cells, such as regulatory T cells, M2 macrophages, and MDSCs.[38,143] Gene expression and immunohistochemical analyses of human HCC revealed that the activated HSC-specific gene expression signatures in peritumoral lesions are an independent risk factor for the poor prognosis of HCC. High expression of activated HSC signature is associated with an immunosuppressive phenotype of infiltrating monocytes/macrophages, suggesting the association of the HSC-macrophage-mediated immunosuppression with the prognosis of HCC patients.[76] The study did not identify the crucial mediators from activated HSCs that promote an immunosuppressive phenotype of macrophages. Other studies have demonstrated the interplay of HSCs and immunosuppressive MDSCs in HCC. In a mouse model of chronic liver injury, p53-deficient HSCs secrete factors that polarize TAMs to the protumorigenic M2 phenotype associated with immunosuppression.[81] MDSCs are an immature myeloid cell population that accumulate in the chronically injured liver and cancer,[39,144] which can suppress the cytotoxic T-cell response.[145,146] The MDSC number is well correlated with HCC tumor size.[147] In humans, MDSCs are known to express CD34,

CD33, and CD15,[148] whereas in mice, MDSCs express both myeloid lineage markers CD11b and Ly6G due to their immature nature.[146] In mice, activated HSCs can induce expansion and accumulation of MDSCs derived from myeloid progenitor cells, which is mediated by IL-6,[149] CD44, complement component 3,[150] COX-2, and catalase-mediated depletion of hydrogen peroxide.[151–154] Also, HSC-derived SDF-1 plays a crucial role in MDSC migration in the mouse HCC model.[155] Based on these findings, preventing MDSC induction and infiltration could be an effective strategy for HCC therapy.

Conclusion

Macrophages and HSCs play pivotal roles in the development of liver fibrosis and HCC. HSCs and liver macrophages in the diseased liver are highly heterogeneous due to various origins and functional plasticity. Recent studies have begun to uncover fibrosis- and cancer-specific phenotypes and subpopulations of HSCs and macrophages and their induction mechanisms. In addition, the interplay of macrophages and HSCs is crucial for HSC activation and fibrosis progression. This interplay can also promote the pre- and promalignant liver microenvironment through ECM remodeling, immunosuppression, SASP, and proinflammatory and profibrogenic cytokines including TGF-β, IL-6, IL-1β, and TNF-α. Further investigations of the molecular mechanism of the HSC–macrophage interplay in fibrosis and HCC might lead to a better understanding of the complicated pathology of fibrosis and HCC and to the development of novel and effective therapies for these deadly liver diseases by targeting both HSCs and macrophages.

Funding

This work was supported by the National Institutes of Health grants R01AA027036, R01DK085252, R21AA025841, and P01CA233452 and by Cedars-Sinai Medical Center (Cedars-Sinai Cancer Center for Integrated Research in Cancer and Lifestyle Award).

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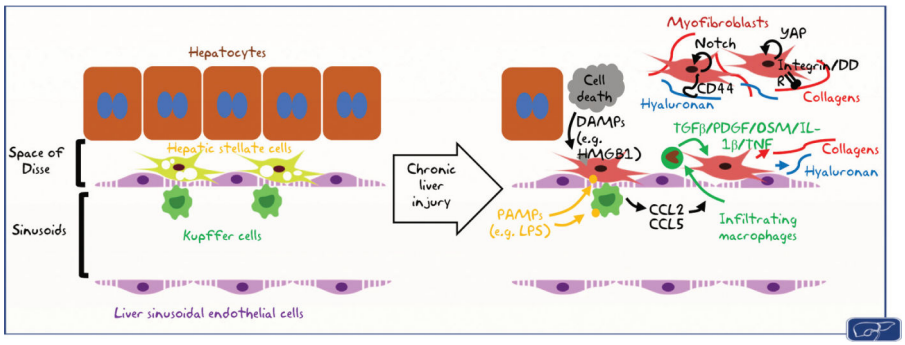

Fig. 1.

The interplay of hepatic stellate cells (HSCs) and macrophages in liver fibrosis: HSCs reside in the space between lined hepatocytes and sinusoids (the space of Disse). HSCs store vitamin A in lipid droplets in the steady state liver (quiescent HSCs). Liver-resident Kupffer cells reside in the intralumen of sinusoids and capture gut-derived molecules. During chronic liver injury, DAMPs (e.g., HMGB1) and PAMPs (e.g., LPS) stimulate HSCs and Kupffer cells to promote the infiltration of bone marrow derived macrophages into the injured site through the production of CCL2 and CCL5. Infiltrating macrophages stimulate HSCs to proliferate and migrate into the injured site and produce extracellular matrix, including collagens and hyaluronan (HA). Collagens and HA further activate HSCs through integrin/DDR and CD44, respectively. Notch and YAP signaling promote HSC activation. CCL2/5, chemokine ligand 2/5; DAMPs, damage-associated molecular pattern; DDR, discoidin domain receptor; IL-1β, interleukin-1β; LPS, lipopolysaccharides; OSM, oncostatin M; PAMPs, pathogen-associated molecular patterns; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; YAP, Yes-associated protein.

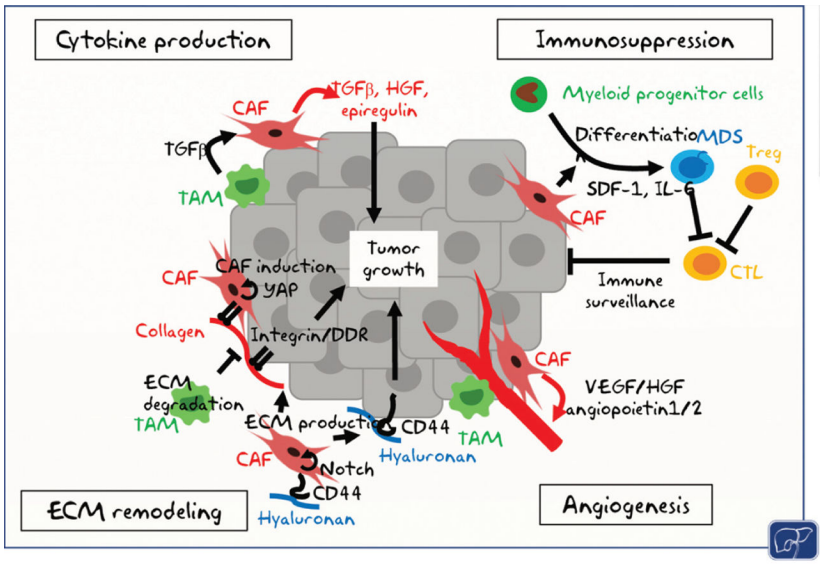

Fig. 2.

The interplay between hepatic stellate cells (HSCs) and macrophages in hepatocellular carcinoma (HCC): graphical summary of key components contained in a unique microenvironment in fibrosis and HCC. The interplay of HSCs, macrophages, and extracellular matrix cooperatively promotes liver fibrosis and HCC. Macrophage-derived cytokines and ECM–integrin-mediated signals could contribute to the differentiation of CAFs from their precursor cells (e.g., HSCs). CAFs could directly promote tumor growth through the production of cytokines and growth factors (e.g., TGF-β, HGF). CAFs and TAMs remodel ECM, including collagens and hyaluronan, which regulate HCC progression through integrins/DDR and CD44-dependent manners. CAFs and TAMs can promote angiogenesis by producing angiogenic factors (e.g., VEGF and HGF). CAFs are associated with the induction of immunosuppressive MDSCs and regulatory T cells, which suppress T cell mediated immune surveillance for HCC. CAF, cancer-associated fibroblasts; CTL, cytotoxic T lymphocytes; DDR, discoidin domain receptor; ECM, extracellular matrix; HGF, hepatocyte growth factor; IL-6, interleukin-6; MDSC, myeloid cell-derived suppressor cells; SDF-1, stromal cell-derived factor 1; TAM, tumor-associated macrophages; TGF-β, transforming growth factor-β; Treg, regulatory T cells; VEGF, vascular endothelial cell growth factor; YAP, Yes-associated protein.

Table 1

Macrophage-derived cytokines/chemokines linked to HSC activation

	Factors	Functions
Cytokines	TGF-β	Primarily produced by macrophages Promotes type I and III collagen production in HSCs through Smad-dependent pathway[42]
	PDGF	Produced by macrophages PDGF-B, PDGF-C, and D/PDGFR- β pathway contributes to fibrosis progression through HSC proliferation and migration[43–48]
	OSM	Produced by macrophages Induces TIMP-1 in HSCs and profibrogenic macrophages, leading to fibrosis progression[117,122]
	TNF-α	TNF- α does not directly induce type I collagen induction in HSCs but contributes to fibrosis by upregulating TIMP-1, downregulating BAMBI, and preventing HSC apoptosis[156–159]
	IL-1β	Produced by proinflammatory macrophages Contributes to fibrosis through an upregulation of TIMP-1 and downregulation of BAMBI in HSCs[105] and HSC survival[118]
Chemokines	CCL2	Produced by macrophages and HSCs Promotes macrophage and HSC infiltration and activation through CCR2[97,160,161]
	CCL5	Produced by macrophages and HSCs Promotes macrophage and HSC infiltration, and HSC activation through CCR1/CCR5[98]

Abbreviations: BAMBI, bone morphogenetic protein and Activin-membrane-bound inhibitor; CCL2/5, chemokine ligand 2/5; CCR1/CCR5, C-C chemokine receptor type 1/5; IL-1β, interleukin-1β; LPS, lipopolysaccharides; OSM, oncostatin M; PDGF, platelet-derived growth factor; PDGFR-β, platelet-derived growth factor receptor β; TIMP-1, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor-α.

Table 2

Biological functions of ECM components in liver fibrosis and HCC

	Fibrosis	HCC
Type I collagen	Activates HSCs and promotes fibrosis through integrin β1/YAP signaling[52] Activates TGF- β through integrin αv signal[53] Induces invasion and proliferation of HSCs through DDR2[55,56] Blocking integrin αv by a small molecule attenuated fibrosis[54]	Promotes HCC proliferation through integrin ß1/FAK signaling in NASH[162] Promotes EMT of tumor cells through DDR2[129,163]
Type IV collagen	Increases in fibrotic livers and consists basement membrane[164,165]	VEGF promotes angiogenesis by regulating type IV collagen receptor, integrin α2β1[166] Arrestin (fragments from type IV collagen) suppresses endothelial cell proliferation, migration, and angiogenesis through integrin αv/β3[167]
Laminin	Supports proliferation and differentiation of hepatic progenitor cells in the fibrotic liver[132,133]	HSC-derived laminin5 stimulates HCC cell migration through the MEK/ERK pathway[134]
Decorin	Acts as antifibrotic by binding to TGF- β and controls its bioactivity[135]	Acts as HCC repressor by interfering PDGFR-α signaling[136]
Hyaluronic acid	Activates HSCs through HA/CD44/Notch1 pathway[57]	Promotes HCC through CD44[137–139]

Abbreviations: DDR2, discoidin domain receptor 2; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; FAK, focal adhesion kinase; HA, hyaluronan; HCC, hepatocellular carcinoma; HSC, hepatic stellate cells; MEK/ERK, mitogen-activated protein kinase/extracellular signal regulated kinase; NASH, nonalcoholic steatohepatitis; PDGFR-α, platelet-derived growth factor receptor α; TGF-β, transforming growth factor-β; VEGF, vascular endothelial cell growth factor; YAP, Yes-associated protein.

Semin Liver Dis. Author manuscript; available in PMC 2021 August 01.