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Fibrosis-Dependent Mechanisms of Hepatocarcinogenesis

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Abstract

Hepatocellular carcinoma (HCC) is a rising worldwide cause of cancer mortality, making the elucidation of its underlying mechanisms an urgent priority. The liver is unique in its response to injury, simultaneously undergoing regeneration and fibrosis. HCC occurs in the context of these two divergent responses, leading to distinctive pathways of carcinogenesis. In this review, we highlight pathways of liver tumorigenesis that depend upon, or are enhanced by fibrosis. Activated hepatic stellate cells drive fibrogenesis, changing the composition of the extracellular matrix. Matrix quantity and stiffness also increase, providing a reservoir for bound growth factors. In addition to promoting angiogenesis, these factors may enhance the survival of both pre-neoplastic hepatocytes and activated hepatic stellate cells. Fibrotic changes also modulate the activity of inflammatory cells in the liver, reducing the activity of natural killer and natural killer T cells that normally contribute to tumor surveillance. These pathways synergize with inflammatory signals, including telomerase reactivation and reactive oxygen species release, ultimately resulting in cancer. Clarifying fibrosis-dependent tumorigenic mechanisms will help rationalize antifibrotic therapies as a strategy to prevent and treat HCC.

Keywords

extracellular matrix; cirrhosis; hepatic stellate cell; natural killer; hepatitis

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, and the third most common cause of cancer mortality (1). In the United States, the incidence of HCC is rising precipitously, primarily as a result of the increasing prevalence of advanced chronic hepatitis C (2), and fatty liver disease (3). The incidence of HCC varies by etiology, race, ethnicity, gender, age, and geographic region, but the presence of fibrosis is a common link among each of these risks (4, 5).

Liver fibrosis is strongly associated with HCC, with ninety percent of HCC cases arising in cirrhotic livers (6). For hepatitis B infection, the presence of cirrhosis, along with age, gender, viral DNA load, and viral core promoter mutations, is a risk factor for HCC (7). Fibrosis has also been identified as a risk factor in hepatitis C infection, where cancer risk is directly related to fibrosis severity (8). Overall, ~80% of hepatitis B and C patients presenting with HCC are already cirrhotic (9). Similarly, HCC development is also linked to alcoholic cirrhosis (10), non-alcoholic steatohepatitis (NASH) (11), and hemochromatosis

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(12), with a yearly HCC incidence of 1.7% in alcoholic cirrhosis (10) and 2.6% in NASH cirrhosis (11).

Despite these associations, the mechanisms linking fibrosis and HCC remain largely unsettled – does fibrogenesis or the presence of fibrosis actively promote HCC, or is fibrosis merely a byproduct of chronic liver damage and inflammation, with no direct impact on tumor formation (Figure 1)? The contribution of inflammation to HCC has been reviewed extensively, and is not the focus of this article; we direct the reader to outstanding articles on NF-κB signaling (13), reactive oxygen species (6, 14), and telomere shortening (15, 16). Here we focus specifically on potential links between fibrosis and HCC.

Fibrosis-Dependent Mechanisms of Hepatocarcinogenesis

While the inflammatory stimuli of fibrosis have been progressively uncovered, these efforts have not yet led to effective antifibrotic therapies. The risk of HCC may be reduced by abrogating the initial, inflammatory insult, but increasing evidence suggests that persistent fibrosis confers its own carcinogenic risk. In other words, clearing hepatitis C in a cirrhotic patient might halt progression of the disease, but may not reduce the risk of HCC. Currently, there is a paucity of clinical data to address this possibility. Potential mechanisms of fibrosis-dependent carcinogenesis include increased integrin signaling by the fibrotic matrix, paracrine signaling between hepatic stellate cells (HSCs) and hepatocytes, increased stromal stiffness, growth factor sequestration by extracellular matrix (ECM), and reduced tumor surveillance by natural killer (NK) and natural killer T (NKT) cells.

Enhanced integrin signaling

Fibrosis is characterized by changes in the amount and composition of ECM components, which contribute to tumorigenesis. Increased deposition of fibrillar collagens types I and III, as well as fibronectin, in hepatic fibrosis provokes cellular responses through the integrin family of transmembrane receptors. When organized into focal adhesions on the cell surface, integrins promote growth and survival by activating the PI3K and MAPK signaling cascades (17). Increased ECM may stimulate integrin signaling in hepatocytes, thereby enhancing the growth and survival of precancerous cells. This prospect is supported by studies that correlate collagen expression, integrin expression, and tumorigenicity in both human HCC samples (18) and mouse HCC models (19). Other proposed mechanisms for integrin-mediated tumorigenesis include increased migration (20, 21)) and enhanced survival through anti-apoptotic signaling (22).

These proposed links between integrin signaling and carcinogenesis do not adequately address the heterogeneity of ligands and signaling between integrins, however. In tumor lines, over-expression of integrin β1 actually leads to growth arrest, attributed to up-regulation of the cyclin dependent kinase inhibitors p21 and p27. In addition, human HCC samples have decreased expression of integrin β3, and its over-expression in a human HCC cell line leads to apoptosis (23). In future studies, the specific ligands and downstream pathways of the integrin heterodimers need to be characterized in both pre-malignant and cancerous cells in order to clarify their combined impact on hepatocarcinogenesis.

In addition to the fibrillar collagens, other ECM molecules including laminin, fibronectin, and several non-fibrillar collagens may also amplify carcinogenic signaling. Although these proteins are in relatively low abundance compared to the fibrillar collagens, their potential function as growth factor receptor ligands could amplify their carcinogenic impact.

Paracrine crosstalk between tumorigenic and stromal cells

Rich intercellular signaling networks exist between tumors and tumor-associated fibroblasts: tumor secretion of PDGF and TGFβ stimulates myofibroblast activation, leading to changes in ECM composition and organization. Reciprocally, activated fibroblasts promote tumor growth and invasion, not only in primary tumors but also in early stages of metastasis (24). This crosstalk has been emphasized in HCC, where stromal gene expression profiles have been correlated with patient survival (25).

As the primary fibrogenic cells in the liver, activated hepatic stellate cells (HSCs) and myofibroblasts may directly support hepatic tumorigenesis. Stellate cells produce growth factors, including HGF, interleukin 6, and Wnt ligands, fostering an environment conducive to hepatocyte proliferation (26). Similarly, hepatic myofibroblasts can enhance the growth and migration of malignant hepatocytes, at least partially through PDGF- and TGFβ mediated mechanisms (27). In addition, hepatic stellate cells secrete more angiopoietin 1 when activated (28), facilitating an angiogenic milieu that is supportive of tumor growth.

Reciprocally, tumors may signal to surrounding stroma. For example, elevated hedgehog signaling has been associated with liver injury in mice and humans (29, 30), and promotes liver regeneration (31). Hedgehog activity has been implicated in the formation and maintenance of malignancies, yet hedgehog ligands fail to drive proliferation in several tumor cell lines. Instead, hedgehog signaling from tumors to the stromal microenvironment may be responsible for promoting tumor progression (32). Since hedgehog signaling may induce epithelial-tomesenchymal transition (33, 34), the tumorigenic effect of hedgehog could be mediated by increased myofibroblast activation and fibrosis. This prospect is supported by a hedgehog antagonist-mediated reduction of myofibroblasts in a mouse model of biliary injury and HCC (35).

Several studies have identified cells resembling activated stellate cells associated with the liver progenitor cell niche, suggesting that these cells may provide paracrine signals that promote stem cell expansion (36). The nature of these paracrine signals, and the mechanisms underlying the supportive role of HSCs in stem cell expansion, are currently unknown and of intense interest.

Increased matrix stiffness

Liver fibrosis increases ECM stiffness, which promotes cell proliferation and HSC activation. Increased stromal stiffness precedes and accompanies fibrosis in chronic liver disease (37, 38), and elevated liver stiffness, as measured by transient elastography, is associated with enhanced risk of HCC (39). Similar paradigms exist in other systems: non-transformed 3T3 cells have increased proliferation on stiff polyacrylamide substrates (40), and enhanced stiffness has been correlated with malignancy in a mouse model of breast cancer (41).

Experimentally, a stiff collagen gel inhibits primary hepatocyte differentiation and promotes proliferation (42). Hepatoma lines are affected similarly, with increased proliferation and chemotherapeutic resistance when grown on increasingly stiff polyacrylamide gels. This effect is mediated by the Fak, Erk, Pkb/Akt, and Stat3 pathways, primarily downstream of integrin β1 signaling (43). Stromal stiffness also increases activation of stellate cells (44) and portal fibroblasts (45), creating a positive feedback loop that continues to promote fibrosis.

Stromal stiffness is regulated in part by matrix metalloproteinases and their inhibitors, but matrix metalloproteinases can regulate cell proliferation independent of their effects on stromal stiffness. Although matrix metalloproteinases degrade the stroma, they paradoxically increase liver growth (46), HSC proliferation (47), and tumor progression (48). Matrix metalloproteinases might also liberate sequestered growth factors (see next section). Alternatively, the production of reactive oxygen species in response to matrix metalloproteinase activity may overcome the loss of stromal stiffness by promoting genomic instability. This type of reactive oxygen species induction is reportedly downstream of an alternatively spliced form of Rac1, which is induced after mammary epithelial exposure to MMP-3 (49, 50). A third possibility is that MMP induction of reactive oxygen species leads to enhanced stellate cell activation, also through a Rac1-mediated mechanism (51).

Growth factor sequestration by the ECM

Growth factors are sequestered by the ECM and signal in an autocrine or paracrine manner to nearby cells (52, 53). Initial work focused on FGF sequestration in the ECM (54), but many other cytokines are passively sequestered, including ligands from the FGF, TGF, BMP, Wnt, and interleukin families. Matrix metalloproteinases can both activate and inhibit growth factor signaling: they liberate growth factors from the ECM, but can also remove the extracellular receptors by cleavage at the cell surface.

Other signaling factors are actively recruited to the ECM by regulatory carrier proteins. For example, TGFβ signaling is highly dependent on ECM interactions. TGFβ is directly recruited to the ECM by latent TGFβ binding proteins (LTBPs), which have affinity for both TGFβ and ECM fibrils. While bound to LTBP, TGFβ is unable to signal. This finding suggests that accumulation of ECM would lead to increased proliferation and decreased apoptosis, since TGFβ signaling would be suppressed. However, LTBPs contain multiple proteinase sensitive sites, and cleavage of those sites by matrix metalloproteinases leads to the release of TGFβ (55).

In the setting of inflammation or increased migratory potential, elevated matrix metalloproteinase activity can liberate sequestered TGFβ. Fibrotic ECM, containing more sequestered TGFβ, would release greater amounts of the cytokine. This could antagonize oncogenesis by inhibiting proliferation and promoting apoptosis. The nature of ECM-cytokine interactions may change depending on the particular cytokine, duration, and cellular context of each interaction. To clarify these relationships, there is a need for controlled, reproducible systems that model the interaction between cells and stroma.

Reduced tumor surveillance by NK and NKT cells

Reduced natural killer (NK) cell function may also contribute to the emergence of HCC in chronic liver disease. NK cells induce apoptosis in cells that have either down-regulated class I MHC expression or up-regulated stress-induced ligands. These expression changes are usually present in tumor cells, allowing NK cells to function in tumor surveillance and control (56). In addition to killing tumor cells, NK cells down-regulate fibrosis by inducing apoptosis of activated stellate cells (57, 58), without affecting quiescent stellate cells (59). Natural killer cells are enriched in the liver (60), but have reduced activity in chronic liver disease (61-63). Fibrosis may inhibit NK cell function by separating them from their tumor and stellate cell targets; NK cells in the tumor microenvironment remain in the stroma, unable to function, instead of making cell-cell contact(64). NK cells express matrix metalloproteinases, and migrate more slowly in the presence of matrix metalloproteinase inhibitors (65), further suggesting that NK function, and subsequently tumor surveillance, is inhibited by the ECM accumulation in fibrosis.

Natural killer T cells are a distinct population of cells that can both direct class switching and induce Fas/perforin-mediated apoptosis (66). Like NK cells, NKT cells home to the liver. CD1d-tetramer^{+}CD4^{+} populations can promote stellate cell activation (67), but CD45R/B220-TCRβ^{+}CD1d-tetramer-reactive iNKT cells areantifibrotic (68). The endogenous activity of NKT cells most likely reflects their level of activation (69). CD1d^{+} and CD3^{+} DX5^{+} NKT cell surveillance of HCC has been established using mouse hepatoma implantation models (70-72), but the effect of fibrosis on NKT tumor surveillance is less clear – although CD1d-tetramer^{+}CD4^{+} NKT cells are increased in the setting of cirrhosis (67) and CD3^{+} Vα24^{+}Vβ11^{+} iNKT cells are increased in hepatic malignancy (73), little is known about their interactions with the ECM.

Conclusions

Several pathways link chronic liver disease, fibrosis, and carcinogenesis (Figure 2), yet a coherent model linking fibrosis to HCC remains elusive. Importantly, key experimental challenges continue to stall therapeutic progress.

Each tumorigenic mechanism may operate across a limited range of the natural history of hepatocellular carcinoma, a concept that can greatly inform the most appropriate models and patients to study. For example, while stromal stiffness promotes cell growth, it only contributes to oncogenesis while cells are unable to proliferate without a stiff stroma. This might be true for pre-malignant hepatocytes, but not tumor cells – carcinoma cell populations have limitless replicative potential and relative independence from extracellular growth signals, allowing them to proliferate independent of stromal stiffness. While stromal stiffness is most likely influential early in the development of HCC, angiogenic factors become increasingly important as solid tumor size increases. It follows that different models or time points should be used for the study of stromal stiffness and angiogenic potential. Many studies focus on cirrhotic patients, presumably because they are easier to define and have more obvious disease phenotypes. This restricts the scope of the study to more advanced pathogenic events. Similarly, molecular studies often use immortalized lines from advanced tumors. Due to the diverse natural history of chronic liver disease, an ongoing

challenge is to identify when particular oncogenic mechanisms are contributing to HCC, and to use experimental models that accurately reflect liver pathology at that point.

To fully clarify the role of fibrosis in HCC development, there is a pressing need for the experimental separation of fibrosis and inflammation, which will facilitate the ability to determine how fibrosis per se contributes to hepatocarcinogenesis. A few existing models may prove useful. For example, a transgenic mouse with hepatic over-expression of PDGF-B (74) leads to activation of hepatic stellate cells, without additional inflammatory stimuli. Alternately, mice expressing collagenase-resistant collagen have delayed fibrosis regression after sustained injury (75), offering the potential to study fibrotic influences after the majority of inflammatory sequelae have resolved. A reciprocal approach would be to induce fibrosis in an immunocompromised animal, although the feasibility of this approach is not established – strong inflammatory stimuli normally accompany myofibroblast activation (76-79).

Models of liver disease are especially lacking in several areas. First, while hedgehog-mediated crosstalk with stroma may facilitate progression in mouse xenograft models (32), the contribution of stromal-tumor hedgehog signaling in the liver is not clear. In addition, no models specifically interrogate the immune defects resulting from fibrosis, which purportedly contribute to HCC – while NK cells contribute to tumor surveillance and their activity is reduced with progressive fibrosis, the actual effect of fibrosis-related NK dysfunction has not been clarified. Lastly, mechanisms linking fibrosis to cancer development in other tissues have been described in breast (41), and several other tumors (32). These may apply to hepatocarcinogenesis, but must be tested directly in liver models.

In hepatocarcinogenesis, the convergence of chronic liver disease, inflammation, and fibrosis is likely to be complex, nuanced, and varied. A recent study reports that liver fibrosis may be protective in the context of acute liver injury (80), suggesting that complete suppression of fibrosis might not be an optimal therapeutic approach. Instead, targeted manipulation of hepatocarcinogenic pathways should be more fruitful. This targeted approach will only be possible with an enhanced understanding of the genetic and epigenetic mechanisms in HCC. Ultimately, a deeper understanding of fibrotic influences will yield valuable insights for the prevention and treatment of hepatic neoplasia.

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Abbreviations

HCC	hepatocellular carcinoma
NASH	non-alcoholic steatohepatitis

HSC	hepatic stellate cell
ECM	extracellular matrix
NK	natural killer
NKT	natural killer T

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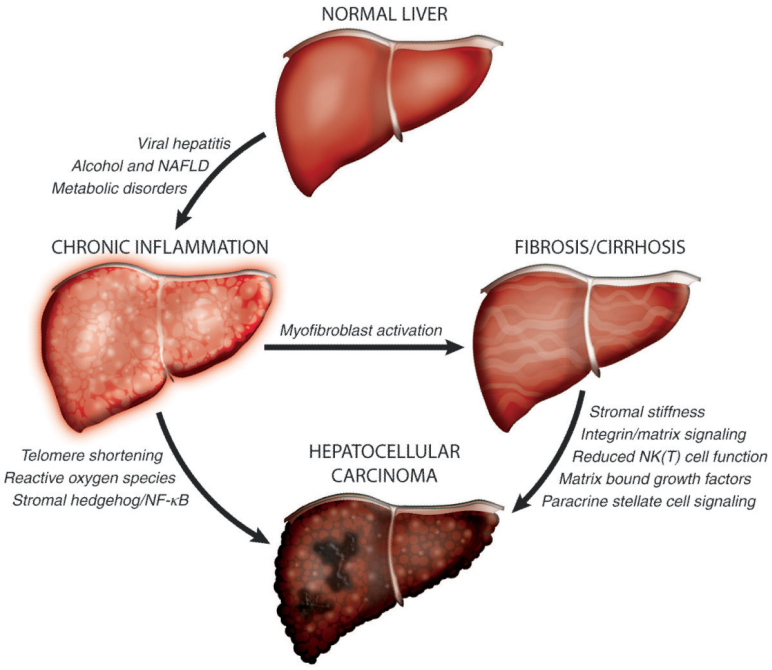

Figure 1.

Fibrosis-dependent and inflammation-related links to HCC. In the liver, chronic infectious, metabolic, or toxic damage leads to persistent inflammation. Chronic damage and inflammation contribute to hepatocarcinogenesis by disrupting telomeres, releasing reactive oxygen species, and altering paracrine signaling in the cellular microenvironment. In addition, chronic inflammation leads to myofibroblast activation, resulting in liver fibrosis and cirrhosis. Fibrosis leads to reciprocal pathways of carcinogenesis: changes in ECM composition and nonparenchymal cell activity ultimately result in a growth-promoting, antiapoptotic environment for hepatocytes. Because fibrosis is intimately associated with chronic inflammation, an ongoing challenge is to experimentally isolate fibrosis-dependent carcinogenic pathways.

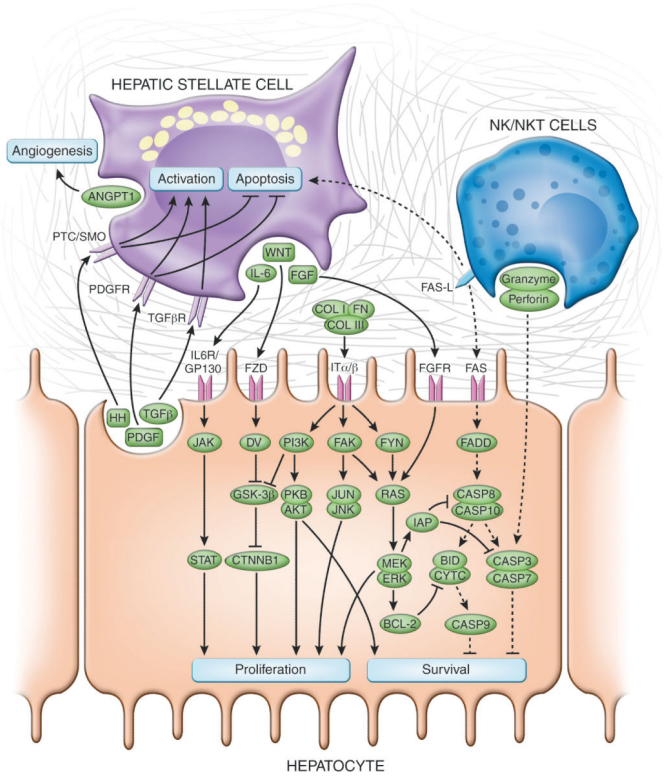

Figure 2.

Pathways in liver fibrosis that promote HCC. Reciprocal signaling between stellate cells and precancerous hepatocytes creates a positive feedback loop, leading to increased hepatocyte growth and HSC activation. HSC activation can also contribute to carcinogenesis by promoting angiogenesis, and by altering the stromal environment. Changes in ECM composition simultaneously promote hepatocyte growth and inhibit NK function. Loss of NK function, in turn, promotes HSC survival and decreases tumor surveillance. Dotted lines indicate pathways suppressed in fibrosis.