Role of integrins in fibrosing liver diseases

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Patsenker E, Stickel F. Role of integrins in fibrosing liver diseases. Am J Physiol Gastrointest Liver Physiol 301: G425–G434, 2011. First published June 9, 2011; doi:10.1152/ajpgi.00050.2011.—Integrins and other cell adhesion molecules regulate numerous physiological and pathological mechanisms by mediating the interaction between cells and their extracellular environment. Although the significance of integrins in the evolution and progression of certain cancers is well recognized, their involvement in nonmalignant processes, such as organ fibrosis or inflammation, is only beginning to emerge. However, accumulating evidence points to an instrumental role of integrin-mediated signaling in a variety of chronic and acute noncancerous diseases, particularly of the liver.

extracellular matrix; hepatic stellate cell/myofibroblast; inflammation; antifibrotic therapy

Liver fibrosis constitutes the uniform response of liver tissue to chronic injury and develops as a consequence of accelerated matrix generation and impaired degradation, partly triggered by chronic inflammation. The present review focuses on established and novel insights into the importance of integrins in the evolution of liver fibrosis and the mediation of inflammation and depicts their potential utility as therapeutic targets in the treatment of viral hepatitis, cholestatic liver diseases, and alcoholic and nonalcoholic fatty liver diseases (NAFLD) and defines several lines of future research focused on possible therapeutic strategies.

Chronic liver diseases may cause persistent inflammation and lead to progressive remodeling due to fibrosis and vascular derangements. Among these diseases, chronic infections with hepatitis B and/or C viruses (HBV and HCV, respectively), persistent alcohol consumption, insulin resistance-associated nonalcoholic steatohepatitis, autoimmune hepatobiliary diseases, and iron overload may all result in liver cirrhosis and failure.

Liver fibrosis and cirrhosis are characterized by an escalating severity of scarring of the liver with a subsequent progressive loss of liver function and an elevated risk of liver cancer. However, successful removal of the causative trigger stops fibrosis progression and carries the potential of substantial fibrosis reversion. However, current therapeutic options to stop fibrosis progression often fail or are implemented too late to accomplish a meaningful impact on liver tissue remodeling. Therefore, direct antifibrotic interventions that intercept with key mechanisms and/or molecules crucially involved in the evolution of fibrosis are required to exert high efficacy to reverse or halt fibrosis development but show good tolerability (18, 61, 65). Unfortunately, the search for such therapies has been largely disappointing so far.

Integrins are a large family of heterodimeric cell surface receptors, which act as mechanoreceptors by relaying the information from cell to cell, and from the extracellular matrix (ECM) to the cell interior and vice versa (29). Since integrin receptors directly bind to components of the ECM and control remodeling thereof, they may play a crucial role in the evolution and progression of liver fibrosis (15).

Surprisingly, scientific reports on targeting integrins in experimental models of fibrosing organ diseases are limited, let alone in humans. However, recent published data point to a pivotal role of certain integrins as important mediators of progressive fibrosis and suggest integrin antagonism as an attractive concept to counteract these processes.

Fibrogenesis

In the liver, the wound healing response to chronic injury is characterized by intense production of interstitial collagens and other ECM components (basement membrane collagens, noncollagenous glycoproteins, proteoglycans, growth factors, etc.) paralleled by a decreased degradation thereof (66). Injury to hepatocytes or bile duct epithelial cells leads to mononuclear cell activation, release of fibrogenic factors, and activation of liver mesenchymal cells. Mesenchymal cells or myofibroblasts include peripoortal and perivenular fibroblasts and hepatic stellate cells (HSC), which, upon activation from various injuries, become the premier source of fibrotic ECM. A central feature of HSC activation leading to enhanced proliferation, contractility, and collagen accumulation is the prominent release of proinflammatory and profibrogenic cytokines such as TGF-β1, PDGF, FGF, connective tissue growth factor, and others. Cytokines and their inhibitors may act in a paracrine manner by recruitment of other cells to join the profibrotic scenario or by sustaining the stimulation of liver cells to continue cytokine release. These can either directly induce the production of fibrous matrix (fibrogenesis) or downregulate the release of matrix-degrading enzymes termed matrix metalloproteinases (MMPs), and upregulate their tissue inhibitors of MMP, which all play a pivotal role in regulating matrix
synthesis (fibrogenesis) and its degradation (fibrolysis). Ultimately, advanced liver fibrosis with liver insufficiency, i.e., decompensated cirrhosis, results from a marked imbalance between fibrogenesis and fibrolysis leading to net matrix accumulation, distortion of the liver vasculature and its autoregulation, and a loss of functional reserves of the liver parenchyma (4, 54, 61).

Current Therapeutic Strategies

Since fibrosing processes are secondary to chronic liver injury from infectious/immunological, toxic, and metabolic causes, current therapeutic strategies aim at eliminating the causative trigger of the disease, for example antiviral therapy for hepatitis B and C (36, 55, 56), immunosuppression for autoimmune hepatitis, abstinence for alcoholic liver disease (1, 14), and endoscopic drainage or surgery for biliary obstructions (24). Although many studies showed that eradication of the trigger that induce fibrogenesis may stop fibrosis progression or even cause fibrosis reversal (64), not all patients respond to treatment of the causative agent, or even reverse fibrosis.

Many potential antifibrotic targets are currently under investigation, including various signaling molecules, growth factors, and receptors mediating autocrine or paracrine activation of profibrogenic effectors (hepatic stellate cells, fibroblasts or inflammatory cells), such as TGF-β1 and its receptor, endothelin A receptors, cannabinoid receptors 1 and 2, interleukins-1, -2, -6 and -8, PDGF, FGF, VEGF, Toll-like receptors, TNF-α, and receptors mediating autocrine or paracrine activation of profibrogenic effectors (hepatic stellate cells, fibroblasts or inflammatory cells), such as TGF-β1 and its receptor, endothelin A receptors, cannabinoid receptors 1 and 2, interleukins-1, -2, -6 and -8, PDGF, FGF, VEGF, Toll-like receptors, TNF-α, and mammalian target of rapamycin (mTOR), phosphodiesterase, MMPs, and others (54, 61, 64). However, despite relatively good effectiveness in cell culture and animal models, only few antifibrotic agents have been tested clinically with regard to their potential to halt or even reverse fibrosis.

Integrin Biology

Integrins are receptors mediating interactions between the cell and their surrounding ECM, and comprise a family of transmembrane cellular protein receptors composed of noncovalently linked α- and β-subunits. These can form at least 24 different combinations that are differentially expressed by various cell types and recognize multiple ligands (Table 1) (25, 40). Intercellular adhesion to ECM via integrins, in addition to growth factor signals, are the major determinants of cell behavior, controlling its fate by regulating proliferation, survival, apoptosis, or differentiation (69). Integrins signal through the cell membrane bidirectionally: 1) the extracellular binding activity of integrins is regulated from the cell interior (inside-out signaling), and 2) the binding of the ECM elicits external signals that are transmitted into the cell (outside-in signaling) (12, 37, 38, 57). Upon binding to the ECM, integrins cluster on the membrane plane and form focal adhesions via recruitment and activation of various signaling and adaptor proteins, such as focal adhesion kinase, Src family of kinases, talin, paxillin, α-actin, tensin, and vinculin (15). Other signals mediated by integrins include changes in intracellular Ca²⁺ concentrations, integrin-linked kinase (ILK) and growth factor signaling pathways, such as the ras-raf-mitogen-activated kinase, protein kinase C, and phosphatidylinositol 3-kinase pathways, depending on the specificity of integrin binding (20, 21, 32) (Fig. 1). In addition to their specific matrix protein ligands, integrins tightly cooperate with growth factors and other functional components present in the extracellular environment. For example, α₁β₆ and α₃β₈ integrins were shown to activate a latent form of TGF-β₁, an extremely potent regulator

<table>
<thead>
<tr>
<th>Integrin Family</th>
<th>Receptor</th>
<th>Protein</th>
<th>Ligand</th>
<th>Reference</th>
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<tr>
<td>β₁</td>
<td>VLA-1 (α₁β₁)</td>
<td>CD49a/CD29</td>
<td>LN, CN</td>
<td>25, 40</td>
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<td>VLA-2 (α₁β₂)</td>
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<td></td>
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<td>ITGA9/CD29</td>
<td>Tenascin, VCAM-1</td>
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<td>LFA-1 (α₂β₃)</td>
<td>CD51/CD29</td>
<td>VCAM-1, –2, –3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(α₂β₃)</td>
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<td>25, 40</td>
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<td></td>
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<td>CD51/CD29</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>LFA-3 (α₂β₇)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>β₃</td>
<td>VNR (α₃β₃)</td>
<td>CD51/CD61</td>
<td>VN, FN, VWF, thrombospondin, CN, tenascin</td>
<td></td>
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<td>(α₃β₃)</td>
<td>CD41a/CD61</td>
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</tr>
<tr>
<td>β₄</td>
<td>(α₄β₈)</td>
<td>CD49f/CD104</td>
<td>LN</td>
<td>40</td>
</tr>
<tr>
<td>β₅</td>
<td>(α₅β₅)</td>
<td>CD51/ITGB5</td>
<td>VN</td>
<td>40</td>
</tr>
<tr>
<td>β₆</td>
<td>(α₅β₆)</td>
<td>CD51/ITGB5</td>
<td>VN, tenascin, LTBP</td>
<td>40, 43</td>
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<tr>
<td>β₇</td>
<td>(α₅β₇)</td>
<td>CD49f/ITGB7</td>
<td>FN, MadCAM-1, VCAM-1</td>
<td>40</td>
</tr>
<tr>
<td>β₈</td>
<td>(α₅β₈)</td>
<td>CD103/ITGB7</td>
<td>E-Cadherin</td>
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</tr>
<tr>
<td></td>
<td>(α₅β₈)</td>
<td>CD51/ITGB8</td>
<td>VN</td>
<td>40</td>
</tr>
</tbody>
</table>

CN, collagen; FG, fibrinogen; FN, fibronectin; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; LN, laminin; LPS, lysophosphatidylserine; LTBP, TGF-β latency-associated protein; MAC, macrophage antigen; MadCAM, mucosal addressin cell adhesion molecule-1; VLA, very late antigen; VN, vitronectin; VNR, vitronectin receptor; VWF, von Willebrand factor.
of ECM expression by HSC (Fig. 2) (41, 43). On the other hand, engagement of /H9251v/H9252 and /H9251v/H9252 upregulates ECM-degrading proteases MMP-2, -3 and -9, which could potentially result in enhanced matrix degradation (7, 30). Changes in particular integrin expression levels on the cell surface may indicate cellular abnormalities or malignant transformation (60). Thus the variability and complexity of integrin-mediated signal transduction pathways underline its universal significance in the regulation of normal and pathological processes.

Many integrins recognize a common motif in their ligands, among which the best studied is the RGD sequence (arginine-glycine-aspartic acid) (13). The integrin-binding activity of adhesion proteins can be reproduced by short synthetic peptides containing this motif. Such peptides promote cell adhesion when unsolubilized onto a surface and inhibit it when presented to cells in solution. Drug design based on the RGD structure may provide new treatments for different diseases in which integrins are involved (8, 62).

Fibrosing Liver Diseases and Integrin Targeting

The expression of integrins from various cell types involved in liver fibrosis development, as well as their capacity to cross talk with growth factors and other signaling molecules render the concept of targeting integrins an attractive approach for antifibrotic therapy. Importantly, clinical studies have found aberrant expression of integrins in patients with chronic liver diseases. For example, in patients with chronic hepatitis C, the expression of /H92521, /H92511, /H92515, /H92516 integrin chains was significantly increased and correlated with histological necroinflammatory activity and stage of fibrosis (46), whereas /H92526 integrin was found to be upregulated in patients with chronic hepatitis B and C, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and alcohol-induced liver injury (53). This implies that integrin overexpression is part of a uniform and coordinated process involved in the progression of liver fibrosis regardless of the underlying etiology. But surprisingly, only few integrin inhibitors have been tested as potential antifibro-

Fig. 1. Integrin inside-out and outside-in signaling pathways. Outside-in signaling: Binding of integrins to extracellular matrix (ECM) leads to integrin clustering on the cell membrane, formation of focal adhesion, and activation of various signaling pathways, depending on specificity of integrin-ligand binding: focal adhesion kinase (FAK) and Src family of kinases, phosphatidylinositol 3-kinase (PI3K), integrin-linked kinase (ILK), growth factor signaling (ras-raf-mitogen-activated kinase), and others. Inside-out signaling: The signals from the cell induce a conformational change of talin, resulting in its binding with the cytoplasmic tail of the β-chain. This binding unclamps the complex between the cytoplasmic α- and β-tails, allowing for high-affinity ligand binding. Apaf-1, apoptotic protease activating factor 1; Casp, Caspase; Grb, growth factor receptor-bound protein; PIPKγ, phosphatatory inositol (4) phosphate 5 kinase type I gamma; PTEN, phosphate and tensin homolog; VCAM, vascular cell adhesion molecule.
tics so far (48, 49, 53), whereas in cancer therapy a number of compounds targeting integrins are currently being tested in phase I and II trials (15). Liver diseases in which integrins were found involved are summarized in Table 2, Fig. 3 displays which and how integrins interact with other regulators of fibrosis development, and Fig. 4 describes current potentially antifibrotic inhibitors.

Viral Hepatitis

The mechanisms by which chronic infection with HBV and HCV damage the liver and, especially, how chronic viral infection drives aberrant hepatic matrix accumulation, are still only partly understood. In this regard, integrins could be crucially involved. An intriguing link between integrins and HCV was found by sequence analysis of the HCV nonstructural protein 3 (NS3) identifying an RGD motif that promotes integrin-mediated cell adhesion within a region of high hydrophilicity and surface probability. Cell adhesion experiments showed that NS3 peptides containing the RGD-sequence bound HUT-78 T-cells in a dose-dependent manner. This suggests that viral proteins such as NS3 disturbs migration of immune cells to infected cells by blocking the RGD-binding site and, thus, protect infected cells from being attacked by the cellular immune response (79). Another study described liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN) as a calcium-dependent lectin that specifically binds naturally occurring HCV in sera of infected patients. This binding was found to be mediated by the HCV envelope glycoprotein E2 and could be blocked by specific inhibitors, including mannan, calcium chelators, and antibodies to the lectin domain of SIGN. Thus L-SIGN seemingly represents a liver-specific receptor for HCV and plays an important role for HCV entry into immune cells and, thus, chronicity of the infection and, possibly, immune tolerance (19).

Cholestatic Liver Diseases

A rather robust body of scientific evidence exists that demonstrates a prominent role of integrin expression and function in the context of chronic biliary injury both in rodents and humans. Thus, in normal liver tissue, bile duct epithelial cells express α2, 3, 5, and 6; connective tissue stroma α1 and 2; and hepatocytes α1 and 5; whereas de novo expression of “bile duct type” α2, 3, and 6 integrins was observed from periportal hepatocytes of patients with cholestatic liver diseases, possibly indicating a phenotypic switch of hepatocytes to bile duct epithelia during persisting cholestasis (71). In liver specimens of subjects with primary biliary cirrhosis, damaged bile ducts

![Diagram](http://ajpgi.physiology.org/)

**Fig. 2.** αvβ6 integrin as an indirect mediator of liver fibrosis progression. Binding of αvβ6/αvβ6 integrin to arginine-glycine-aspartic acid (RGD) sequence of latency-associated protein (LAP) from TGF-β1 large latent complex elicits the dissociation of a latent form of TGF-β1 and release of active TGF-β1. Activated TGF-β1 then exerts its profibrotic activity via binding to TGF-β1 receptor (TGFBR1) on mesenchymal cells or hepatic stellate cells (HSC).

**Table 2. Integrins and integrin chains expression in various liver diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Integrins</th>
<th>Cell Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV</td>
<td>RGD-binding integrins, VLA-1, −5, −6, LFA-1, αv, L-SIGN</td>
<td>hepatocytes, endothelial cells</td>
<td>19, 79</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>α1, α2, α6, ICAM/LFA-1, VCAM/VLA-4, VLA-2, αvβ6</td>
<td>peripoortal hepatocytes, cholangiocytes, neutrophils</td>
<td>16, 23, 31, 33, 44, 48, 53, 68, 71, 72, 75, 76</td>
</tr>
<tr>
<td>ALD</td>
<td>VLA-3, −4, −5, −9, β1, ICAM-1, β2</td>
<td>T-lymphocytes, hepatocytes, neutrophils</td>
<td>3, 5, 6, 10, 11, 59, 63, 73</td>
</tr>
<tr>
<td>NAFLD</td>
<td>β1</td>
<td>muscle cells</td>
<td>83</td>
</tr>
<tr>
<td>Inflammation</td>
<td>α4β2, LFA-1, MAC-1, p150,95, VLA-4, CD44, β1</td>
<td>B and T lymphocytes, natural killer cells, monocytes</td>
<td>2, 8, 9, 17, 22, 26, 34, 47, 52, 67, 77</td>
</tr>
</tbody>
</table>

IR, insulin resistance; L-SIGN, liver/lymph node-specific intercellular adhesion molecules-3 grabbing nonintegrin; VCAM, vascular cell adhesion molecule.
were found to express intercellular adhesion molecules (ICAM)-1, and a proportion of bile duct epithelial cells expressed vascular adhesion molecule (VCAM)-1. On infiltrating lymphocytes surrounding bile ducts lymphocyte function-associated antigen (LFA)-1 and very late antigen (VLA)-4 were detected, whereas in control livers these alterations in antigen expression on the bile ducts were either not observed or were only focal and substantially weaker. These findings suggest that ICAM-1/LFA-1 and also VCAM-1/VLA-4 linkages between the damaged bile ducts and lymphocytes may facilitate antigen-specific reactions to the peri-ductal lymphocytes in PBC (75, 76).

Other experimental studies in mice demonstrated that expression of αβ3 integrin on cholangiocytes confers susceptibility to rotavirus (RV)-induced experimental biliary atresia (31, 44). Integrin blocking assays using monoclonal antibodies and short-interfering RNA led to a significant reduction in attachment and yield of virus in RV-infected cholangiocytes. A recent experiment using the same animal model of biliary atresia in mice showed that αβ6 integrin is markedly upregulated in this type of liver injury (44). In line with this observation, the αβ6 receptor was suggested as a marker of biliary fibrosis. In fact, its blockage with specific antagonist prevented the progression of biliary fibrosis via inhibition of TGF-β1 activation, cholangiocyte proliferation, peribiliary collagen deposition, and downregulation of profibrogenic cytokines and led to improved liver architecture and function (48, 53, 72). Even a single dose of the αβ6 inhibitor injected into fibrotic Mdr2(Abcb4)−/− mice significantly induced profibrolytic MMP-8 and -9 after 3 h, with a corresponding increase in ECM-degrading activities (53).
suggesting that CD4+ T lymphocytes with upregulated CD11a receptor play a role in Th-1-predominance within the autoimmune process of PBC (68). Neutrophils may aggravate acute liver injury via β2-dependent extravasation of neutrophils from sinusoids and reactive oxygen formation, and mice knocked out for β2 that underwent bile duct ligation (or β1-naphthylisothiocyanate) toxicity had significantly fewer neutrophils and plasma transaminase activities and significantly less hepatic necrosis compared with wild-type mice (23, 33). Another study demonstrated that targeting LFA-1 (β2-integrin) on neutrophils with anti-LFA-1 antibody decreased serum transaminase levels, reduced leukocyte adhesion in postsinusoidal venules and restored sinusoidal perfusion in cholestatic mice, indicating that LFA-1 may exert significant protection against pathological inflammation and liver damage in cholestatic liver diseases (16).

These findings suggest a therapeutic potential of integrin inhibitors in acute or chronic biliary injury.

Alcoholic Liver Diseases

In human alcoholic liver diseases (ALD), a significantly higher expression of CD29 (β1-integrin), VLA-3, VLA-4, and VLA-5 was observed on T lymphocytes (CD2+CD8+CD4+ cells) compared with control subjects, whereas no changes were observed for CD18 (β2-integrin) and CD50 (ICAM-3) expression (63). Other studies revealed an enhanced expression of the β1-integrin on hepatocytes membranes in a large percentage of patients with different stages of ALD (11), and the serum level of β3-integrin was increased in patients with advanced alcoholic liver fibrosis or cirrhosis (73).

Infiltration of the liver with neutrophils is a prominent feature of alcoholic hepatitis in both experimental animals and humans. It was shown that transmigration of neutrophils can be mediated by a chemokine gradient established toward the hepatic parenchyma, which involves the interaction between adhesion molecules: β2-integrins on neutrophils and ICAM-1 on endothelial cells. Following transmigration, neutrophils adhere to sensitized hepatocytes through β2-integrins and ICAM-1 and mediate the decay of hepatocytes mostly by oxidative stress and proteases (59). In patients with ALD, ICAM-1 expression on hepatocytes was significantly greater in alcoholic steatohepatitis (ASH) compared with fatty liver and normal controls and correlated with the histological degree of hepatocellular damage, parenchymal inflammation, and LFA-1 expression on parenchymal leukocytes. Thus the ICAM-1/LFA-1 pathway may be involved in

<table>
<thead>
<tr>
<th>Structure of the compound</th>
<th>Affinity and specificity</th>
<th>Disease application</th>
<th>Reference</th>
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<tr>
<td>RGD mimetics (nonpeptidic)</td>
<td>adhesion to FN – percent inhibition (100µg/ml)</td>
<td>Con A-induced hepatitis, TAA-induced liver fibrosis</td>
<td>8</td>
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<tr>
<td>SF-6.5</td>
<td>56%</td>
<td>Con A-induced hepatitis</td>
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<td>NS-11</td>
<td>61%</td>
<td></td>
<td></td>
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<td>αβ6 integrin antagonist (nonpeptidic)</td>
<td>IC50</td>
<td>FN</td>
<td>48</td>
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<td></td>
<td></td>
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<td>α4β7 inhibitors (peptidic)</td>
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<td>CRSDTLCGE-NH2</td>
<td>0.03 µM</td>
<td>Spontaneous colitis</td>
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<td>CRSDTLC-NH2</td>
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<tr>
<td>RGD-peptides</td>
<td></td>
<td>1 mg/kg body weight</td>
<td>CCl4-induced liver fibrosis</td>
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</table>

Fig. 4. Integrin inhibitors in liver diseases. IC50, the concentration of the compound necessary to inhibit binding of the receptor to immobilized ligand (FN) to 50% of control; BDL, bile duct ligation; ConA, concanavalin A; TAA, thioacetamide; MadCAM, mucosal addressin cell adhesion molecule.
leukocyte-mediated tissue damage during alcoholic hepatitis (10). In rats, chronic alcohol administration also induces the expression of CD18 (β2-integrin) adhesion molecules on neutrophils, enhancing neutrophil migration, as well as releasing chemotactic cytokines and growth factors by Kupffer cells, whereas the use of an anti-CD18 antibody attenuates hepatic injury in vivo (5, 6). In addition, neutrophil infiltration into the liver parenchyma was shown to be mediated by osteopontin via α5β1 and α2β1 integrins, which may be responsible for the more pronounced liver injury in a rat model of ASH (3).

Nonalcoholic Fatty Liver Diseases

Dynamic analysis of glucose homeostasis in muscle-specific integrin β1-deficient mice demonstrated a marked increase of insulin resistance as reflected by a significant reduction of insulin-stimulated glucose infusion rate and glucose clearance (83). Whole body insulin resistance results from a specific inhibition of skeletal muscle glucose uptake and glycogen synthesis without any significant effect on insulin suppression of hepatic glucose output or insulin-stimulated glucose uptake in adipose tissue. These in vivo data demonstrate a crucial role of integrin β1 in the evolution of insulin resistance, a condition considered a hallmark of NAFLD and nonalcoholic steatohepatitis. To what extent other integrins may be involved in the pathogenesis of NAFLD, for example in triggering inflammation or fibrosis, is not known. Considering the obvious importance of integrin β1 in the evolution of insulin resistance, studies investigating the effect of antagonizing integrin β1 on the natural course of the metabolic syndrome and its hepatic manifestation, NAFLD, seems therefore worthwhile.

Inflammation

The invasion of liver tissue by immunoactive cells contributes to the pathogenesis of human liver diseases and consecutive fibrosis. In this respect, the characterization of the composition of the mononuclear infiltrate surrounding affected biliary canaliculi in tissue sample of patients with PSC revealed a significant excess of infiltrating B and T lymphocytes positive for integrin α4β7 (52). This integrin plays an essential role in lymphocyte trafficking throughout the gastrointestinal (GI) tract via interaction with mucosal addressin cell adhesion molecule (MAdCAM). Elevated intestinal and hepatic MAdCAM expression has been linked to GI disorders (Crohn’s disease, ulcerative colitis, and hepatitis C) (17). In patients with inflammatory bowel disease and PSC, blocking antibodies to MAdCAM-1, α4β7, or the integrin α4-chain inhibited adhesion of αβ7+ lymphocytes to MAdCAM-1 expressed on hepatic vessels, providing a mechanism to explain why the hepatic recruitment of mucosal lymphocytes in inflammatory liver disease can complicate inflammatory bowel disease, and indicating α4β7-selective antagonists as GI-specific anti-inflammatory agents (22).

An elevated CD11a (αL), CD11b (αM), CD11c (αX), and CD49d (α4) integrin expression was observed on peripheral blood leukocytes in liver cirrhosis, which further increased along with the progression of liver failure, whereas blockade of CD11b and CD44 diminished monocyte localization to hepatic foci (47, 67). Investigating the role of inducible CD11b in the regulation of natural killer (NK) cells revealed that neutralizing CD11b-antibody enhanced cytotoxicity, interferon-γ, and granzyme B production of Toll-like receptor 3 triggered NK cells, and in vivo depletion of NK cells and adoptive transfer of CD11b-deficient NK cells attenuated experimental acute hepatitis (77). CD16(+) monocytes were found to be recruited to the liver via a combination of adhesive signals involving VAP-1 and CX3CR1-mediated integrin β1 activation, allowing them to localize at sites of chronic inflammation and fibrogenesis (2). The chemokine CXCL16 has been reported to promote lymphocyte adhesion to epithelial cells via conformational activation of β1-integrins and binding to VCAM-1, alleviating attraction and retention of effector cells that promote biliary and hepatocyte destruction in inflammatory liver disease (26). Again, a number of studies showed that the use of synthetic analogs of the RGD motif prevented acute and chronic experimental liver injury and fibrosis (8, 9, 34). Thus, in the rodent model of concanavalin A-induced acute hepatitis, elevation of serum transaminases and TNF-α and the infiltration of liver tissue by inflammatory cells were inhibited by pretreatment of mice with the synthetic RGD mimetic. In rats, the progression of thioacetamide-induced cirrhosis was markedly inhibited by the coadministration of the RGD mimetic SF-6-5, and in carbon tetrachloride (CCL4)-induced liver fibrosis, RGD peptide (GRGDS) improved liver fibrosis via inhibition of collagen production and acceleration of collagenase activity.

Targeting Hepatic Stellate Cells

Myofibroblasts or HSC, as the main source of ECM, could be affected directly via integrins expressed on their surface, irrespective of the etiology of liver disease, influencing their deactivation, apoptosis, or interactions with other cells and even phagocytosis. Accordingly, lymphocyte phagocytosis by HSC was observed and was completely prevented by blocking ICAM-1 and integrin molecules (αL) (42).

HSCs were shown to express VLA-1, -2, -5, integrins mediating cell adhesion to collagens and fibronectin, which can even modulate the expression of fibrolytic enzyme MMP-1 (27, 58, 82). Thus HSC activation increases α3β1 integrin expression and interactions of α3β1 with ECM increase collagen production via actin cytoskeletal reorganizations, as well as via activation of Src kinases and ERK/JNK signaling molecules families (39). Interestingly, connective tissue growth factor, a promoter of liver fibrosis, was found to regulate integrin expression in primary culture HSC and support HSC adhesion via binding of its cell surface integrin α3β1, which interacts cooperatively with heparan sulfate proteoglycans or fibronectin (27). De novo expression of α8β1 in activated HSC during hepatic injury due to either CCl4 injury or bile duct ligation in rats was also demonstrated (35). ILK was found to be strongly induced in CCL4-induced rat liver fibrosis and colocalized with α-smooth muscle actin of HSC residing in perisinusoidal areas, affecting HSC spreading and migration (78). Another study described the inhibition of migratory capacity of HSC, induced by PDGF-BB, TGF-β1, and collagen I by α1- and α2-integrin blocking antibodies (74).

An interesting observation has been reported regarding α3β3 integrin. It was found to be upregulated in HSC during activation and its antagonists significantly inhibited adhesion, proliferation, as well as procollagen I synthesis of HSC plated on normal collagen (28, 50, 80, 81). However, its pharmacoe-
tical blockade in different experimental animal fibrosis models aggravated fibrosis, most possibly because of the high expression of $\alpha_\beta_3$ on activated endothelial cells, resulting in consecutive impairment of angiogenesis and liver regeneration (49).

**Future Perspectives**

Despite expanding experimental research into integrins and their impact on fibrotic liver diseases, many gaps of knowledge must be closed to decipher their implication in the development of antifibrotic treatments. In particular, knowledge derived from in vitro and in vivo experimentation awaits translation into the clinical situation. Several RGD peptides and nonpeptidic RGD mimetics showed good efficacy in alleviating liver fibrosis in animal models, but whether these results can be reproduced in humans remains to be tested. So far, none of the described integrin-targeting compounds have been tested in trials with fibrosis as the primary end point, but some have been studied in humans for other indications including some cancers. In addition to research in humans, integrin-related studies of fibrosis in animal models should be expanded to oncology issues with a special focus on treatment and prevention. As for the liver, hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) could be amenable to integrin-targeted therapies since both malignancies either express integrins ($\alpha_\beta_3$ expression from CC cells) or depend on structures (blood vessels) that may respond to anti-integrin interventions ($\alpha_\beta_3$ on vascular endothelial cells). Above all, prevention of cirrhosis per se represents effective hepatoma prevention since most HCCs evolve in cirrhotic livers, so any therapy that retards or reverses fibrosis progression will likely reduce hepatoma incidence. For example, TGF-$\beta$ signaling plays a decisive role in both hepatic fibrosis and CC as evidenced by upregulated $\alpha_\beta_3$ integrin in both conditions.

A note of caution must be placed when applying integrin-targeting compounds with antiangiogenic activity, such as $\alpha_\beta_3$ integrin inhibitors (cilengitide), in fibrotic patients with liver tumors, since they may result in inadvertent biological outcomes with consecutive liver failure due to their impact on cell/tissue regeneration and function. Thus identification and further refinement of compounds specifically targeting HSC/myofibroblasts and their triggers of activation are warranted that interrupt with key events of fibrosis evolution, but simultaneously sustain the integrity of other liver-protective conditions (cell proliferation, angiogenesis, immune surveillance).

**Conclusion**

Integrins are clearly implicated in the evolution of liver fibrosis, and their targeting therefore represents an attractive concept of therapeutic intervention, particularly because experimental data suggest potent efficacy of such an approach. However, more work is needed to identify possibilities to specifically antagonize integrins (including RGD mimetics, specific integrin antagonists, or small nonpeptidic molecules) to halt or reverse fibrosis and avoid detrimental effect of such inhibition. It should be kept in mind that integrin receptors are expressed on numerous cell types and mediate an abundance of physiological processes modulating angiogenesis, tissue repair, immune activity, and cell renewal. Therefore, potential benefits must be weighed against so far unknown adverse effects.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


INTEGRINS AND LIVER FIBROSIS


