Stromal cell-derived factor-1 (SDF-1) as a target in liver diseases

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CHEMOKINES ARE SMALL SECRETED proteins that bind to G protein-coupled receptors on their target cells. In humans, there are 48 chemokines known that ligate to 19 different chemokine receptors. They can be roughly divided by their function into homeostatic and inflammatory chemokines (63). Homeostatic chemokines are constitutively expressed in specific tissues or cells and participate in organogenesis, development, and stem cell migration (63). Inflammatory chemokines are induced during inflammation to attract target cells carrying their cognate receptor to sites of injury. Inflammatory chemokines play exceptional roles in the pathogenesis of liver diseases including orchestrating immune reactions in the liver upon injury, controlling the progression of hepatic fibrosis as well as promoting or limiting the development of hepatocellular carcinoma (HCC) or liver metastases (37). One of the most interesting chemokines with versatile functions in the liver is the chemokine CXCL12 that is also known as pre-B-cell-growth-stimulating factor (PBSF) or stromal cell-derived factor 1 (SDF-1), owing to its initial cloning from a bone marrow-derived stromal cell line. CXCL12 is broadly expressed under healthy conditions throughout the body, specifically by different immune cells, endothelial cells, and stem cells. In the liver environment, CXCL12 is produced by biliary epithelial cells, hepatic stellate cells (HSC), and liver sinusoidal endothelial cells (LSEC) but also by malignant cells (37). However, results from recent studies using genetic tracking and highly pure cell isolation techniques questioned CXCL12 synthesis by biliary epithelial cells, hepatic stellate cells (HSC), and liver sinusoidal endothelial cells (LSEC) and tumor cells mainly respond via CXCR4. The CXCL12 synthesis by tumor cells is consistent with evidence from experimental models of liver diseases in animals and thorough analyses of human samples from patients with liver diseases indicate that CXCL12/SDF-1 could represent an attractive target for novel therapeutic interventions in various settings of liver injury and cancer.

Expression of the SDF-1 Receptors CXCR4 and CXCR7 in the Liver

CXCL12 was first identified as a signal from the bone marrow microenvironment (“stromal cell-derived factor,” SDF-1) to regulate hematopoiesis, including B lymphopoiesis (39). In search of the receptor for CXCL12, it became apparent that Cxcr4−/− mice had hematopoietic and cardiovascular abnormalities strikingly similar to those of Cxcl12−/− mice.

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sorafenib as the primary physiological receptor for CXCL12 (64). CXCR4 belongs to the family of seven transmembrane G protein-coupled receptors (GPCR). CXCR4 is constitutively expressed in whole liver tissue and primary hepatocytes (58), as well as on stem cells, T and B lymphocytes, monocytes, neutrophils, dendritic cells, and HSC. With respect to functional relevance in liver homeostasis and injury responses, the CXCR4 expression by HSC and their striking phenotypic changes upon CXCL12 activation appear most prominent (26). Upon ligand binding to the receptor, a conformational change triggers the subsequent intracellular signaling events involving ERK1/2, MAPK, JNK, and AKT pathways (19). Cytoskeletal rearrangements, actin polymerization, polarization, pseudopodia formation, and adhesion processes are a hallmark of the ligand-stimulated chemotaxis (52). As detailed below, malignant hepatocytes can express both CXCL12 and CXCR4, suggesting that this pathway could act as an autocrine signal to stimulate cancer cell growth, migration, and invasion (53).

Recently, it has become evident that another receptor, CXCR7, is able to interact with CXCL12 (1, 4). CXCL12 can bind to this atypical chemokine receptor, also termed ACKR3, even with a greater affinity than to CXCR4 (11). Because of its inability to initiate GPCR signaling, CXCR7 was initially thought to act as a scavenger receptor for CXCL12 (40). Later, it was shown that this receptor signals via the β-arrestin-2 pathway (42). Activation of the CXCR7 signaling pathway increases cell proliferation and adhesion properties (4). In the liver, CXCR7 seems to be very important on LSEC (13). More recently, CXCR7 was found on circulating LSEC-progenitor cells as well, emphasizing the relevance of this receptor for vascular homeostasis and angiogenesis in the liver (12). Similar to CXCR4, high CXCR7 expression has been found on hepatoma cell lines and metastatic HCC samples. Thereby, CXCR7 might contribute to HCC growth and invasiveness via activation of MAPK and angiogenesis signaling pathways (32).

Transgenic mouse models revealed that the deletion of either CXCL12, CXCR4, or CXCR7 is embryonically lethal (18, 39, 54). These results underline the crucial importance of this chemokine axis in development. Thus, before this chemokine axis can be considered for therapeutic targeting in liver diseases (currently available data are summarized in Table 1), a thorough understanding of the distinct interplay between CXCL12 and its main receptors in the pathogenesis of hepatic disorders is crucial.

The SDF-1 Axis in Acute Liver Injury and Regeneration

Data from patients on the specific role of CXCL12 in acute liver diseases are scarce. However, this pathway has been investigated in mouse models, indicating a central role of CXCL12 in modulating regeneration from acute hepatic insults (Fig. 1A). In response to acute liver damage in mice, caused by a single injection of carbon tetrachloride (CCl4) or an overdose of acetaminophen, CXCR7-expressing LSECs are activated by CXCL12 to initiate liver regeneration (13). After CCl4 administration, hepatocyte proliferation decreased, and the extent of liver injury was aggravated in mice with an endothelial cell-specific knockdown of CXCR7 (13). Therefore, CXCR7 seems to be proregenerative in acute liver injury. Similarly, rats undergoing partial hepatectomy rapidly upregulate CXCL12 expression in liver and LSECs, which increases the proliferation of CXCR7+ LSEC precursors in the bone marrow, stimulates their mobilization into the circulation, and promotes their engraftment in the hepatic sinusoids (12). Interference with either CXCL12 or CXCR7 impairs liver regeneration (12).

Expression of CXCL12 gradually increases after a single injection of mice with CCl4 and peaks at 21 days after the initial injury, concurrent with mesenchymal stem cell (MSC) homing to the damaged liver (22, 35). Recruitment of CXCR4-expressing hematopoietic progenitors in response to CXCL12 was demonstrated to be an important mechanism for liver tissue repair (29). CXCL12 levels roughly double after partial hepatectomy in the liver of rats, resulting in correspondingly increased plasma levels of CXCL12 (12). Vascular endothelial

Table 1. Therapeutic approaches addressing the CXCL12/CXCR4/CXCR7 pathway (selected examples)

<table>
<thead>
<tr>
<th>Therapeutic Agent</th>
<th>Company (Trade Name)</th>
<th>Target(s)</th>
<th>Disease</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD3100 (Plerixafor)</td>
<td>Genzyme (Mozobil)</td>
<td>CXCR4 inhibitor</td>
<td>fibrosis</td>
<td>no improvement, enhanced inflammation (46)</td>
</tr>
<tr>
<td>TC14012</td>
<td>R&amp;D Systems</td>
<td>CXCR7 agonist</td>
<td>ischemia/reperfusion</td>
<td>improved hepatic recovery (58)</td>
</tr>
<tr>
<td>AMD070</td>
<td>ChemoCentryx</td>
<td>CXCR4 inhibitor</td>
<td>partial hepatectomy</td>
<td>no improvement (58)</td>
</tr>
<tr>
<td>NOX-A12</td>
<td>Noxxon</td>
<td>CXCL12 inhibitor</td>
<td>fibrosis</td>
<td>reduction of fibrosis (13)</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Bayer AG (Nexavar)</td>
<td>multiple kinases</td>
<td>fibrosis</td>
<td>no impact (10)</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Bristol-Myers Squibb (Opdivo)</td>
<td>PD-1 antibody</td>
<td>no data available on liver diseases yet</td>
<td></td>
</tr>
<tr>
<td>Sorafenib + AMD3100</td>
<td></td>
<td></td>
<td>no data available on liver diseases yet</td>
<td></td>
</tr>
<tr>
<td>Sorafenib + AMD3100 + Nivolumab</td>
<td></td>
<td></td>
<td>HCC (human)</td>
<td>ClinicalTrials.gov: NCT00105443; approved standard therapy for advanced HCC (3); development of resistance (6, 44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCC (human)</td>
<td>ClinicalTrials.gov: NCT01658878 (ongoing), promising initial results (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCC</td>
<td>inhibition of tumor growth, preventing increased tumor fibrosis (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCC</td>
<td>inhibition of tumor growth and lung metastases, regression of established tumors (9)</td>
</tr>
</tbody>
</table>

Summary of available pharmacological inhibitors of CXCL12, CXCR4, and CXCR7 as well as standard and novel HCC therapeutics.
growth factor (VEGF) appears to be an upstream inducer of CXCL12 expression in the liver (12). Conversely, microRNA-27b suppresses the directional migration of MSCs by downregulating CXCL12 protein expression, thereby counteracting liver repair (35). This microRNA is reduced in CCl4-injured mouse liver (35).

Data from animal models suggest a protective role also for CXCR4 in the late regression phase after acute liver injury. Pharmacological inhibition by AMD3100, a specific inhibitor of CXCR4, does not improve hepatic injury after CCl4 injection but rather increases necrosis and infiltrating inflammatory cells (46). CXCR4 and CXCL12 are both upregulated after hepatic ischemia-reperfusion injury as well. Contrarily, in this model treatment of mice with AMD3100 resulted in increased hepatocyte proliferation and reduced necrosis after the reperfusion. Similarly, administration of recombinant CXCL12 decreased the proliferation of hepatocytes after injury (58). These results imply that the CXCR4/CXCL12 axis mediates the hepatic regeneration after the ischemic injury. On the basis of further findings, the authors propose that CXCR4 antagonism leads to mobilization of a vasculogenic cell population from the bone marrow, which resulted in improved angiogenesis and recovery of the ischemic tissue. Interestingly, after partial hepatectomy the CXCL12/CXCR4 axis was indispensable for hepatic regeneration, suggesting a specific mechanism following oxygen deprivation (58).

Modulation of Hepatic Stellate Cell Activation and Liver Fibrosis by SDF-1-Dependent Pathways

A number of studies from human patients suggested an involvement of SDF-1-dependent pathways for the progression of chronic liver diseases and hepatic fibrosis (Fig. 1B). CXCL12 is upregulated in endothelial cells associated with inflammatory foci and is significantly elevated, compared with controls, in the plasma of patients with advanced liver fibrosis due to chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection (56). In this early study, CXCR4 expression...
was detected on a large fraction of hepatic lymphocytes of HBV/HCV-infected patients, suggesting a function of CXCL12 for endothelial-lymphocyte interactions (56). Similarly, CXCL12 and CXCR4 expression is increased in hepatitis C patients with cirrhotic livers. The authors of this study could demonstrate that CXCL12 binds to CXCR4 on HSC, thereby inducing HSC proliferation and collagen production perpetuating fibrosis (26). Besides CCR5, CXCR4 is a coreceptor for T-tropic (X4) HIV-1 entry (16). In HIV/HCV coinfected livers, the HIV-1 X4-envelope protein gp120 elicits profibrotic effects on activated human HSCs in a CXCR4-dependent fashion (25). Moreover, CXCL12 was found to promote HSC contraction in a CXCR4-dependent but calcium-independent manner by using HSC cell lines and primary human HSC (45). Given the fundamental role of HSC activation for liver fibrosis progression (51), these studies suggest that specific targeting of CXCR4 might be beneficial in patients with chronic liver diseases.

Therefore, several mouse models have been investigated to dissect the differential contribution of CXCL12, CXCR4, and CXCR7 to chronic liver injury and to explore therapeutic interventions in these preclinical models. Using mice with cell type-specific deletions of CXCR7/CXCR4 as well as prototypic models of liver fibrosis (repetitive CCl4 injections and surgical bile duct ligation), a loss of CXCR7 during chronic liver injury and concomitant upregulation of CXCR4 in LSEC were found to cause progression to fibrosis in the liver (13). Thus the proregenerative CXCR7 pathway and the profibrotic CXCR4-pathway balance liver regeneration and fibrosis, supporting the hypothesis that inhibiting CXCR4 might ameliorate fibrogenesis. In line with the above findings, CXCR4 and CXCL12 expression increases in livers of fibrotic mice (46). But, surprisingly, treatment of these mice with AMD3100 either once weekly or continuously via an osmotic pump did not improve hepatic fibrosis and even worsened inflammation (46). This finding was strengthened by another study demonstrating that AMD070, an orally bioavailable inhibitor of CXCL12/CXCR4, did not alleviate hepatic fibrosis in the CCl4 model in mice (10). Furthermore, the conditional ablation of CXCR4 (using the MxCre-system in conditionally targeted Cxcr4 flox/flox mice) revealed that CXCR4-deficient mice were more susceptible to severe chronic liver damage induced by repetitive CCl4 injections (55).

During chronic liver injury, CXCL12 appears to be important to recruit MSC to the injured livers, which might partly explain contradictory results on this pathway for fibrosis and repair processes. In the model of repetitive CCl4 injections in mice, the CXCL12/CXCR4 pathway was the key chemotactic axis regulating MSC migration from the bone marrow to the liver, and adoptive transfer of MSC aggravated early liver fibrosis in this model (34).

Taken together, the SDF-1 axis is upregulated in patients with chronic liver diseases and is functionally involved in the progression of liver fibrosis. However, simply blocking profibrotic CXCL12/CXCR4 did not suffice in ameliorating hepatic fibrosis in vivo, likely owing to off-target effects on the proregenerative functions of the SDF-1 axis. Thus more cell type-, context-, or function-specific strategies to modulate CXCL12/CXCR4 signaling in liver fibrosis are warranted to target these pathways in chronic liver diseases.

Impact of SDF-1 Pathways on Growth and Invasiveness of Liver Cancer

The CXCL12/CXCR4/CXCR7 axis received growing attention, as it became evident that targeting this pathway might be beneficial for many types of cancers (52). For instance, malignant cells expressing CXCR4 and CXCR7 are prone to metastatic spread to organs expressing high levels of CXCL12 such as the liver, where they then seed secondary tumors (52). This mechanism is involved in the development of liver metastases in several types of cancer, including breast, lung, or pancreatic cancer (52) as well as gastrointestinal stroma tumors (57). Because of the important involvement of CXCL12 and CXCR4/CXCR7 in liver homeostasis and regeneration (see above), the CXCL12 axis is of critical relevance for primary hepatobiliary cancers (Fig. 1C) as well (15).

Several studies that investigated the expression of either CXCL12 or CXCR4 on liver resections from patients with HCC unequivocally reported upregulation of this pathway in patients with HCC (15, 31, 48). In a three-dimensional HCC invasion culture model, CXCR4 was gradually increased at the early and intermediate stages of HCC invasion and decreased at the late stage, while CXCL12 was dramatically and continuously upregulated during the entire invasion process (7). Indeed, expression of CXCR4 has been suggested as a prognostic marker for HCC survival (41). In a cohort of 86 HCC patients, intermediate and high CXCR4 levels predicted a poor overall survival. Furthermore, a reduced CXCL12 expression was found in tumor tissues compared with peritumoral tissues. Interestingly, in this study, no prognostic relevance was detected for CXCR7 expression, even though a migration of HCC cell lines in response to CXCL12 was confirmed in vitro both for CXCR4 and CXCR7 (41). Similarly, CXCR4 expression was an independent risk factor for lymph node and bone metastasis in HCC patients (59, 60). In fact, invasion of tumor cells to the lung, lymph nodes, and bone marrow are the predominant routes for HCC metastasis (3). These observations were confirmed by a recent meta-analysis, which demonstrated that 1) CXCR4 is overexpressed in HCC tissues but not in normal hepatic tissue and is higher in HCC than in cirrhosis; 2) expression levels of CXCR4 do not increase during local progression; however, CXCR4 expression increases the risk of distant metastases in HCC; and 3) high levels of CXCR4 gene expression are associated with worse survival in HCC (27). Similar to the role in HCC, high CXCR4 expression was also found to be associated with tumor progression, metastasis, and poor outcome in 122 patients with intrahepatic cholangiocellular carcinoma (62).

Mechanistically, a predominant function of the CXCL12/CXCR4 axis in tumorigenesis is to induce metastasis. This was demonstrated in vitro by using recombinant CXCL12 and cancerous ascitic fluid from HCC patients, which both induced metastatic migration (33). Besides the detrimental involvement in the spreading of cancer cells, the CXCL12/CXCR4 axis also promotes angiogenesis. The expression of CXCR4 in cultured mononuclear phagocytes and several cancer cell lines is strongly induced under hypoxic conditions, a process that involves the transcription factor HIF-1 (49). This accounts also for CXCL12, as its upregulation in ischemic tissue was reported as well (5). There is further a close cross talk between CXCL12 and other signals favoring neangiogenesis, such as
VEGF (12, 20). Another interesting aspect of how the tumor microenvironment influences CXCL12/CXCR4 was uncovered in mice deficient for matrix metalloproteinase-10 (MMP10) that were subjected to a liver cancer model. MMP10-deficient mice with diethylnitrosamine-induced HCCs showed a reduced expression of CXCR4 in livers during hepatocarcinogenesis, alongside smaller and fewer liver tumors, reduced tumor vascularization, and fewer lung metastases compared with wild-type controls (17). These findings, which were confirmed in vitro using human HCC cells, indicate that MMP10, besides other factors such as transforming growth factor-β, hypoxia, and oxidative stress, can drive CXCR4 expression in HCC cells (17).

Role of the SDF-1 Axis for Myeloid-Derived Suppressor Cells During HCC Development: Implications for New Combination Therapies?

Myeloid-derived suppressor cells (MDSC), which are characterized as Gr1+CD11b+ myeloid cells in mice, are a heterogeneous population of immune cells of myeloid origin. They can emerge from blood, bone marrow, or spleen and increase during cancer, infections, or inflammation (21). MDSC regulate immune responses by suppressing T cell functions and are therefore thought to directly promote tumor metastasis and to inhibit antitumoral T cells responses (21). In humans, the existence and function of MDSCs in HCC patients was initially described in 2008 (23). Two modes of action for CD8+ T cell suppression by MDSC were suggested: the suppression of T cell responses through increased arginase activity, which inhibits T cell proliferation, and the induction of regulatory T cells (23). To date, it is known that the suppressive mechanisms used by the different subsets of this heterogeneous population strongly depend on the underlying pathology (21). The comprehensive comparison of different mouse models of HCC revealed that the accumulation of MDSCs is a late event during hepatocarcinogenesis and is highly dependent on the tumor model used (28). Tumor-infiltrating MDSC express CXCR4 and, when isolated from tumor tissue, migrate in response to recombinant CXCL12 in vitro. A neutralizing antibody against CXCR4 is able to inhibit this process (61). Interestingly, a certain portion of MDSCs can also express CXCR7, but the involvement of this receptor in directed migration of MDSCs is currently not fully understood (61). Several specific inhibitors of either CXCL12, CXCR4, or CXCR7 are being developed, and it has been suggested that their prime value could be to sensitize patients to conventional or emerging novel cancer therapies (14). For instance, NOXA12 is an RNA oligonucleotide in L-configuration (“Spiegelmer”) that binds and neutralizes CXCL12 and was shown to interfere with chronic lymphocytic leukemia cell motility and cause chemosensitization (24).

In the field of liver cancer, the multikinase inhibitor sorafenib is still the approved standard therapy for advanced HCC (3). However, HCC mortality rates remain high, partly due to acquired resistance to this antiangiogenic drug (6, 44). The group of Dan Duda was able to demonstrate that HCC treatment with sorafenib increases hypoxia and CXCL12 expression in a model of orthotopic HCA-1 tumor seeding in immunocompetent C3H mice subjected to CCI4-induced liver fibrosis (8). Increased CXCL12 expression led to the accumulation of tumor-promoting (proangiogenic and immune-suppressive) inflammatory cells. Strikingly, a simultaneous therapy with AMD3100 inhibited HCC growth, a finding that could be attributed to the immune-suppressive effects of the tumor microenvironment, mediated by MDSC (8). In a follow-up study, they showed that further addition of an antibody directed against the immune checkpoint inhibitor programmed death-1 (PD-1) significantly decreased tumor growth (9). Thus the CXCL12/CXCR4 pathway mediates the establishment of an immunosuppressive microenvironment and contributes to systemic disease progression, especially after treatment with antiangiogenic agents, like sorafenib or regorafenib.

The therapeutic approach of modulating immune checkpoint pathways, e.g., by targeting T cell inhibitory molecules, like PD-1, PD-L1 (PD-ligand-1), or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), represents a major breakthrough for the immune therapy of solid tumors, because it can prevent the tumor’s escape from cytotoxic T cell responses (36, 50). In an early phase clinical trial, immunotherapy of patients with advanced HCC with the anti-PD-1 antibody nivolumab resulted in durable responses and promising overall survival in a substantial fraction of HCC patients. The 12-mo survival rate exceeded 60% in patients in whom sorafenib had failed (30). Larger trials inhibiting PD-1/PD-L1 interactions by nivolumab or pembrolizumab in HCC have been initiated. However, it is known that not all patients of a certain cancer entity respond to these immunological checkpoint antagonists (2, 43). It is therefore conceivable that the addition of anti-CXCL12 agents (e.g., AMD3100, NOX-A12, or CXX2066) could, via their effects on HCC growth, angiogenesis, and tumor-associated MDSC development, serve as important sensitizers to a successful immune checkpoint inhibitor therapy.

Conclusions

The SDF-1 axis is upregulated in patients with various liver diseases, and mouse models indicate a complex interplay between CXCL12 and CXCR4- or CXCR7-expressing cells in the pathogenesis of hepatic disorders. In acute liver injury, CXCL12 and CXCR7 seem to play a major role in liver regeneration by promoting hepatocyte proliferation. Additionally, the CXCL12/CXCR4 pathway is essential for recruiting stem cells from the bone marrow to sites of injury to assist in tissue regeneration. Although the administration of recombinant CXCL12 could augment repair processes, the regenerative function of CXCL12 appears to depend on interactions with CXCR7, while CXCL12-CXCR4 activation can also be detrimental for liver regeneration. Similar findings were obtained from chronic injury and fibrosis, where CXCL12-CXCR4 interactions can activate profibrogenic stellate and mesenchymal cells. Surprisingly, the simple inhibition of CXCR4 in mouse models was not sufficient to ameliorate chronic hepatic damage. In HCC development, a contribution of CXCR4 is clearly evident, as its expression correlates with poor patients’ survival. Cancer cells hijack the CXCL12/CXCR4 axis during metastases to migrate to distant sites and to establish new tumors. Moreover, CXCL12 promotes a pro-tumorigenic microenvironment characterized by angiogenesis and MDSC suppressing antitumoral immunity. A selective anti-CXCR4 or anti-CXCL12 therapy might ameliorate cancer progression and improve prognosis. Indeed, combination of the
standard anti-HCC drug sorafenib with a CXCR4 blocker in experimental mouse models is able to inhibit HCC tumor growth, therefore emerging as an interesting new approach for HCC therapy. More insights into cell type-, context-, or disease-specific roles of SDF-1 signaling are warranted to develop strategies to modulate CXCL12/CXCR4 signaling in chronic diseases.

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**AUTHOR CONTRIBUTIONS**

A.L. and F.T. prepared figures; A.L. and F.T. drafted manuscript; A.L. and F.T. approved final version of manuscript.

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