Hepatocellular carcinoma and CXCR3 chemokines: a narrative review

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Abstract

Hepatocellular carcinoma (HCC) results from several factors like viral hepatitis infection [hepatitis B, or C (25%)] or occupational exposure. T-helper (Th1) inflammatory cells, characterized by interferon (IFN)-γ and interleukin (IL)-2 secretion, predominate in the liver during chronic HCV infection, and chemokines attracting these cells are particularly important in disease progression. Among C-X-C chemokines, the non-ELR group [as IFN-γ-induced protein 10 (IP-10), monokine induced by IFN-γ (MIG) and IFN-inducible T-cell-alpha chemoattractant (I-TAC)], attracts Th1-cells interacting with chemokine C-X-C receptor (CXCR3). IP-10 has uniquely been shown to have prognostic utility as a marker of treatment outcome. IFN-γ-induced chemokines, as MIG and IP-10, may promote lymphocyte recruitment to HCC playing important roles in cancer immunology. The production of CXC chemokines by HCC cell lines has been shown. It has been identified immune-gene signature that predicts patient survival including the chemokine gene IP-10. Inflammatory cytokines (tumour necrosis factor-α, IFN-γ) and Toll-like receptor 3 ligands stimulate intratumoral production of these chemokines which drive T and Natural Killer cells tumor infiltration, leading to enhanced cancer cell death. Furthermore selective recruitment of CXCR3(+) B-cells that bridges proinflammatory IL-17 response and protumorigenic macrophage polarization in HCC has been shown, suggesting that blocking CXCR3(+) B-cell migration or function may help defeat HCC. It has been also shown that the overexpression of IP-10, which induced by liver graft injury, may lead to cisplatin resistance via ATF6/Grp78 ER stress signaling pathway in HCC; IP-10 neutralizing antibody could be a potential adjuvant therapy to sensitize HCC-cisplatin treatment.


Key words: hepatocellular cancer, IP-10, occupational hepatocellular cancer, Th1 chemokines

State of the Art

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, and it is one of the most common tumors worldwide. It occurs most commonly in countries where hepatitis B infections are common (1), for example in sub-Saharan Africa, central and Southeast Asia, and the Amazonia. Males are usually more affected than females and it is most common between the age of 30 to 50 (2).

HCC causes 662,000 deaths worldwide per year (3). Most cases of HCC result from a viral hepatitis infection [hepatitis B, or C (25%)], metabolic toxins such as alcohol or aflatoxin, conditions like hemochromatosis and alpha 1-antitrypsin deficiency or nonalcoholic steatohepatitis (NASH) (4). In countries where hepatitis B and C are endemic, they are the predominant cause of HCC (5). Whereas in countries where Hepatitis B and C are rare, the major cause of HCC is cirrhosis (often due to obesity or alcohol abuse).

HCC can be caused by the occupational exposure to viruses or chemicals. Toxic hepatitis cases were reported in workers exposed to dimethylformamide, dimethylacetamide, or trichloroethylene (TCE). Viral hepatitis was chiefly reported among health care workers such as doctors, nurses and clinical pathology technicians who could easily be exposed to blood. Preventive measures for these groups therefore include vaccination and serum monitoring programs.

TCE as an industrial pollutant may damage human health and can be considered as carcinogen (6-9), and its metabolites, e.g., trichlorethanol, trichloro-acetic acid and epoxides were recently identified as strong mutagens. Histological alterations, as depletion of glycogen and hydropic degeneration, are present in the liver, however, other signs of TCE effects can be found also in various organs. TCE induces predominantly HCC.

Vinyl chloride monomer is a known cause of angiosarcoma of the liver. It also has other toxic effects on the liver, and it has recently been suggested that exposure to vinyl chloride also causes HCC (6-9).

Since hepatitis B or C is one of the main causes of HCC, prevention of this infection is key to then prevent HCC (10). In the case of patients with cirrhosis, alcohol consumption is to be avoided. HCC may directly present with yellow skin, loss of appetite, weight loss, abdominal pain especially in the right upper quadrant, nausea, vomiting (11). In most cases HCC occurs in people who already have symptoms of chronic liver disease and presents either with worsening of symptoms or during surveillance. People at risk (for example with chronic hepatitis C or B) need to be screened with
ultrasound (US) every 6 months. Alpha-fetoprotein (AFP) is a marker that is useful if it is markedly elevated. In people with a higher suspicion of HCC (such as rising AFP) the best method of diagnosis involves a computed tomography (CT) scan of the abdomen. Triple phase helical CT improves the detection of these tumors. An alternative to a CT imaging study would be Magnetic Resonance Imaging (MRI). MRI has about the same sensitivity for detecting HCC has helical CT, biopsy is not needed to confirm the diagnosis of HCC if certain imaging criteria are met. The prognosis of HCC is affected by the staging of the tumor as well as the vessels function due to the effects of liver cirrhosis (12). Treatment options for HCC and prognosis are dependent on many factors but especially on tumour size, staging, and extent of liver injury. Tumor grade is also important; high-grade tumors will have a poor prognosis, while low-grade tumors may go unnoticed for many years, as is the case in many other organs. While surgical resection offers the best chance at a cure for HCC, the tumors are often inoperable due to large size or spread into vascular and adjacent structures. Medical management is generally palliative and aimed at reducing liver disease symptoms. Chemotherapy is traditionally ineffective. Interventional radiology offers minimally invasive treatments that can improve quality of life, increases survival, and reduces symptoms in these patients. HCC in patients with chronic infections of hepatitis B and/or C can aid the development of the disease by repeatedly causing the body’s own immune system to attack the liver cells (13). While this constant cycle of damage followed by repair can lead to mistakes during repair which in turn lead to carcinogenesis. In chronic hepatitis B, however, the integration of the viral genome into infected cells can directly induce a non-cirrhotic liver to develop HCC. Alternatively, repeated consumption of large amounts of ethanol can have a similar effect. The toxin aflatoxin from certain Aspergillus species of fungus is a carcinogen and aids carcinogenesis of hepatocellular cancer by building up in the liver.

Cytokines and chemokines are very important in the pathogenesis of HCC. The objective of this narrative review is to evaluate the importance of chemokine (C-X-C motif) receptor (CXCR)3, chemokine (C-X-C motif) ligand (CXCL)10/interferon (IFN)-γ-induced protein 10 (IP-10) in HCC. The presentation of data has been reported according to the International Narrative Systematic Assessment (INSA) tool (14).

**IP-10 in inflammatory disorders**

The chemokine IP-10 is able to regulate inflammation acting on several levels. In fact it induces integrin activation, generates directional migration of multiple cell types, including activated T cells, monocytes, and natural killer (NK) cells (15). Additionally IP-10 induces apoptosis of pancreatic beta cells and inhibits the proliferation of both epithelial and endothelial cells (16, 17). Other IP-10 proinflammatory functions are: induction of molecules, as interlukin (IL)-8 and CXCL5, and up-regulation of costimulatory cell surface molecules, as CD54, CD80, and CD86, on monocytes. IP-10 secretion is dependent on interferon (IFN)-γ, which is itself mediated by the IL-12 cytokine family. Several cell types (such as T lymphocytes, monocytes, splenocytes, fibroblasts, keratinocytes, thyrocytes, preadipocytes), under cytokines influence, contribute to IP-10 secretion. Then the detection of high level of IP-10 in peripheral liquids is a marker of host immune response, especially T helper (Th)1 orientated T-cells.

High production of IFN-γ and tumor necrosis factor (TNF)-α may be due to the recruitment of Th1 lymphocytes. Inflammatory cytokines in turn stimulate IP-10 secretion from the above mentioned cells, therefore creating an amplification feedback loop (18).

Circulating levels of IP-10 are increasing with age. Recently some studies showed that the serum and/or the tissue expressions of IP-10 are increased in organ specific autoimmune diseases (19), such as type 1 diabetes (T1D) (20), Graves’ disease (GD), or Graves’ ophthalmopathy (GO) (21-23), autoimmune thyroiditis (24-29), systemic rheumatological disorders like rheumatoid arthritis (RA) (30), systemic sclerosis (SSc) (31-33), psoriasis or psoriatic arthritis (34-38), sarcoidosis (39, 40), hepatitis C virus (HCV)-related cryoglobulinemia (41-45), other HCV immune mediated disorders (46, 47), lupus (48, 49), and also in cancers (50-58).

**Hepatocellular carcinoma, and CXCR3 chemokine IP-10**

A first study (59) examined serum CXC chemokine levels in HCC patients and demonstrated the production of CXC chemokines by HCC cell lines. It was determined the effect of both HCC patient serum and tumor cell conditioned supernatant upon lymphocyte expression of chemokine receptor CXCR3 as well as lymphocyte migration. Lastly, it was examined the chemotactic responses of lymphocytes derived from HCC patients. This study demonstrated functional desensitization of the chemokine receptor CXCR3 in lymphocytes from HCC patients by CXCR3 ligands secreted by tumor cells. This may cause lymphocyte dysfunction and subsequently impaired immune defense against the tumor (59).

A second study (60) aimed to estimate the correlation between mRNA expression of chemokines and tumor-infiltrating lymphocytes in 44 HCC patients. Significant close correlations were observed between the number of infiltrating lymphocytes in these HCC patients and the expression of monokine induced by IFN-γ (MIG) and IP-10 mRNA. In the immunostaining, expression of MIG and IP-10 proteins was found only in the HCC cells in the high-infiltration group. These data suggested chemokines induced by IFN-γ, such as MIG and IP-10, may promote lymphocyte recruitment to HCC and may thus play important roles in cancer immunology (60).

As Th1 inflammatory cells, characterized by IFN-γ and IL-2 secretion, predominate in the liver during chronic HCV infection, chemokines that attract these cells might be particularly important in disease progression. Among the CXC chemokines, the non-ELR group comprised of IP-10, MIG and IFN-inducible T-cell-alpha chemotactrant (I-TAC), attracts Th1 cells through the interaction with their receptor, CXCR3. IP-10 has uniquely been shown to have prognostic utility as a marker of treatment outcome (61).

A further study (62) aimed to investigate the effects...
of adiponectin on liver cancer growth and metastasis and explore the underlying mechanisms. Tumor growth was significantly inhibited by adiponectin treatment, accompanied by a lower incidence of lung metastasis. Tumor vascular endothelial cell damage was found in the treatment group. In vitro functional study showed that adiponectin not only downregulated the ROCK/IP-10/VEGF signaling pathway but also inhibited the formation of lamellipodia, which contribute to cell migration (62).

A study (63) aimed to determine the independent contribution of factors including IL-28B polymorphisms, IP-10 levels and the homeostasis model assessment of insulin resistance (HOMA-IR) score in predicting response to therapy in chronic hepatitis C (CHC). Multivariate analysis of factors predicting rapid (RVR) and sustained (SVR) vireological response in 280 consecutive, treatment naive CHC patients treated with peginterferon alpha and ribavirin in a prospective multicentre study was done. The results showed that in HCV genotype 1 patients, IL-28B polymorphisms, HCV RNA load and IP-10 independently predict RVR (63).

A further study (64) evaluated the ability of immune genes expressed in the tumor microenvironment to predict disease progression. The identified immune-gene signature that predicts patient survival includes the chemokine genes CXCL10, CCL5 and CCL2, whose expression correlates with markers of Th1, CD8(+) T and NK cells. It was suggested that inflammatory cytokines (TNF-α, IFN-γ) and Toll-like receptor 3 (TLR3) ligands stimulate intratumoral production of these chemokines which drive tumor infiltration by T and NK cells, leading to enhanced cancer cell death (64).

The importance of IP-10 in the pathogenesis of HCC is also reviewed in another study (65).

Patients with HCC receiving living donor liver transplantation appear to possess significantly higher tumor recurrence than the recipients receiving deceased donor liver transplantation. The underlying mechanism for HCC recurrence after transplantation remains unclear.

A study (66) aimed to investigate the impact of small-for-size liver graft injury on HCC recurrence after transplantation. The results showed that post-transplant enhanced IP-10/CXCR3 signaling in small-for-size liver grafts directly induced endothelial progenitor cell (EPC) mobilization, differentiation and neovessel formation, which further promotes tumor growth. It was suggested targeting IP-10/CXCR3 signaling may attenuate early-phase liver graft injury and prevent late-phase tumor recurrence/metastasis after transplantation (66).

Interferon lambda 4 (IFN-λ4) is a novel type-III interferon that can be generated only in individuals carrying a ΔG frame-shift allele of an exonic genetic variant (rs368234815-ΔG/TT). The rs368234815-AG allele is strongly associated with decreased clearance of HCV infection. A study (67) explored the biological function of IFN-λ4 expressed in human hepatic cells-a hepatoma cell line HepG2 and fresh primary human hepatocytes (PHHs). In PHHs, secreted IFN-λ4 induced expression of the IP-10 transcript and a corresponding pro-inflammatory chemokine, IP-10. In IFN-λ4-expressing HepG2 cells, decreased proliferation and increased cell death were also observed. All IFN-λ4-induced phenotypes-activation of interferon-stimulated genes (ISGs), decreased proliferation, and increased cell death—could be inhibited by an anti-IFN-λ4-specific antibody (67).

B cells consistently represent abundant cellular components in tumors; however, direct evidence supporting a role for B cells in the immunopathogenesis of human cancers is lacking, as is specific knowledge of their trafficking mechanisms. A study (68) demonstrated that CXCR3(+) B cells constitute approximately 45% of B-cell infiltrate in human HCC and that their levels are positively correlated with early recurrence of HCC. Depletion of B cells significantly suppresses M2b polarization and the protumorigenic activity of tumor-associated macrophages and restores the production of antitumorigenic IL-12 in vivo. The results of this study suggest that selective recruitment of CXCR3(+) B cells bridges proinflammatory IL-17 response and protumorigenic macrophage polarization in the tumor milieu, and blocking CXCR3(+) B-cell migration or function may help defeat HCC (68).

Tumor recurrence remains an obstacle after liver surgery, especially in living donor liver transplantation (LDLT) for patients with HCC. The acute-phase liver graft injury might potentially induce poor response to chemotherapy in recurrent HCC after liver transplantation. A study (69) intended to explore the mechanism and to identify a therapeutic target to overcome such chemoresistance. It was shown that the overexpression of IP-10, which induced by liver graft injury, may lead to cisplatin resistance via ATF6/Grp78 ER stress signaling pathway. IP-10 neutralizing antibody could be a potential adjuvant therapy to sensitize cisplatin treatment (69).

Discussion

Most cases of HCC result from viral hepatitis infection (hepatitis B, or C [25%]), that can be due also to occupational exposure. Th1 inflammatory cells, characterized by IFN-γ and IL-2 secretion, predominate in the liver during chronic HCV infection, and chemokines that attract these cells are particularly important in disease progression. Among the CXC chemokines, the non-ELR group comprised of IP-10, MIG and I-TAC, attracts Th1 cells through the interaction with their receptor, CXCR3. IP-10 has uniquely been shown to have prognostic utility as a marker of treatment outcome. It has been shown the production of CXC chemokines by HCC cell lines. It has been identified immune-gene signature that predicts patient survival including the chemokine gene CXCL10. It was suggested that inflammatory cytokines (TNF-α, IFN-γ) and TLR3 ligands stimulate intratumoral production of these chemokines which drive tumor infiltration by T and NK cells, leading to enhanced cancer cell death. Furthermore selective recruitment of CXCR3(+) B cells that bridges proinflammatory IL-17 response and protumorigenic macrophage polarization in HCC has been shown, suggesting that blocking CXCR3(+) B-cell migration or function may help defeat HCC. It has been also shown that the overexpression of IP-10, which induced by liver graft injury, may lead to cisplatin resistance via ATF6/Grp78 ER stress signaling pathway in HCC.

In conclusion, IFN-γ-induced chemokines, as MIG and IP-10, may promote lymphocyte recruitment to HCC playing important roles in cancer immunology, and IP-10...
neutralizing antibody could be a potential adjuvant therapy to sensitize cisplatin treatment of HCC.

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