The role of cytokines in hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is a frequent malignancy worldwide with a high rate of metastasis. The hepatitis B and C viruses are considered major etiological factors associated with the development of HCC, particularly as a result of their induction of chronic inflammation. There is increasing evidence that the inflammatory process is inherently associated with many different cancer types, including HCC. Specifically, this review aims to cover evidence for the potential roles of cytokines, an important component of the immune system, in promoting HCC carcinogenesis and progression. A global summary of cytokine levels, functions, polymorphisms, and therapies with regard to HCC is presented. In particular, the role of proinflammatory Th1 and anti-inflammatory Th2 cytokine imbalances in the microenvironment of HCC patients with metastasis and the possible clinical significance of these findings are addressed. Overall, multiple studies, spanning many decades, have begun to elucidate the important role of cytokines in HCC. J. Leukoc. Biol. 80: 1197–1213; 2006.

Key Words: liver · inflammation · immune · metastasis · hepatitis

INFLAMMATION AND CANCER

The immune system is composed of a large variety of cells and mediators that interact in a complex and dynamic network to protect the host against foreign pathogens and to simultaneously maintain tolerance toward self-antigens. This system is categorized into innate and adaptive immunity, both of which are key participants in generating acute and chronic inflammatory responses. Innate immune cells, such as macrophages, NK cells, and dendritic cells (DC) are the first line of defense against a foreign pathogen. These cells maintain tissue homeostasis by continuously monitoring the environment for signs of distress and are critical for the activation and modulation of the specific adaptive immune response. The adaptive immune cells such as T cells express antigen-specific receptors and generate diverse antigen recognition, stimulating B cells and ensuing acute or chronic responses dependent on the duration of the antigenic stimulus. Activated, adaptive immune cells and other immune-related components in turn activate other cells of the immune system and thereby enhance the inflammatory response to enable pathogen clearance.

In most instances, the inflammatory response is beneficial to the host; however, when tissue homeostasis is chronically perturbed, interactions between innate and adaptive immune cells can be disturbed. Altered interactions between the innate and adaptive immune cells can lead to chronic inflammatory disorders. The failure to appropriately engage and/or disengage the immune system can lead to excessive tissue remodeling, loss of tissue architecture from excessive cell growth, apoptosis/necrosis, and oxidative stress-related protein and DNA alterations. Under some circumstances, these effects can lead to an increased risk of cancer development.

The association between immune cells and cancer has been known for quite some time [1]. The relationship between these processes is currently the subject of intensive research and is a wide-ranging and complex association as evidenced by the greater than 17,000 manuscripts found in a PubMed literature search for “inflammation AND cancer.” It was initially thought that leukocyte infiltrates around a lesion represented an attempt by the host immune system to eradicate the tumor cells. This is true in some cases, such as the observed favorable prognosis associated with excessive infiltration of macrophages and NK cells in human gastric and colon carcinomas [2, 3]. However, in many other malignant tissues, such as human breast carcinoma and lung adenocarcinoma, infiltrates of macrophages and mast cells, respectively, have been associated with an unfavorable prognosis [4–8]. Population-based studies have shown that individuals who are prone to chronic inflammatory disorders have an increased risk of cancer development [9]. In fact, genetic polymorphisms that encode crucial immune-related genes have been identified as etiological factors in chronic inflammatory disorders. In addition, 15% of human cancers are associated with infectious agents such as Helicobacter pylori and hepatitis viruses, which can promote carcinogenesis through the induction of chronic inflammatory states [10]. In immune-competent mice, which lack key mediators of host immune defense such as IFN-γ or GM-CSF, the spontaneous development of various types of cancer has been observed in tissues that exhibit low levels of chronic inflammation [11]. Epidemiological studies report that inhibiting chronic inflammation in patients with premalignant disease or in those who are predisposed to cancer development is chemopreventive. For example, anti-inflammatory drugs such as aspirin or selective cyclooxygenase 2 inhibitors can significantly reduce

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One component of the immune system that molds the host response to foreign pathogens is cytokines, which are secreted or membrane-bound proteins that regulate the growth, differentiation, and activation of immune cells. Cytokines are released in response to a diverse range of cellular stresses including infection, inflammation, and carcinogen-induced injury. These proteins are generally grouped into two categories, proinflammatory or anti-inflammatory, although several cytokines do not fit specifically into either category. In fact, many cytokines have pleiotropic functions, and some can act in a synergistic manner. Several cytokines, particularly those produced by CD4+ Th cells, are defined as Th1 or Th2 and are comprised mainly of ILs. Th1 cytokines (e.g., IL-1α, IL-1β, IL-2, IL-12p35, IL-12p40, IL-15, and non-ILs, e.g., TNF-α and IFN-γ) are generally referred to as proinflammatory, and the Th2 cytokines (e.g., IL-4, IL-8, IL-10, and IL-5) induce anti-inflammatory responses. Cytokines normally function to stimulate a host response aimed at controlling cellular stress and minimizing cellular damage. The failure to resolve an injury can provoke excessive immune cell infiltration and lead to persistent cytokine production. Therefore, the host response to stress provokes changes in cytokine expression, which can impact several stages of cancer formation and progression.

The liver hosts many cell types that are susceptible to the actions of cytokines. Hepatocytes bear a variety of cytokine receptors such as IL-1, TNF-α, and IL-6. Nonparenchymal cells, such as the resident liver macrophages (Kupffer cells), not only synthesize many cytokines, but the actions of these immune cells can also be affected by the cytokine environment. Liver sinusoidal endothelial cells are also targets and producers of various cytokines. NKT cells are generally increased in chronically infected livers and produce profibrotic cytokines (IL-4 and IL-13) [14, 15]. Cytokines have therefore been implicated in liver development and regeneration but may also contribute to the pathogenesis of liver-related diseases such as cirrhosis, fibrosis, and cancer.

The effect of cytokine function on tumorigenesis and progression is complex as a result of the pleiotropy and apparent redundancy of cytokine action. Despite this, many experiments have begun to elucidate the complex associations between cytokines and tumors. In this review, we discuss the emerging evidence in which cytokines play a role in hepatocellular carcinoma (HCC) and suggest that manipulation of cytokine balance can potentially be exploited for cancer therapy.

**INFLAMMATION AND HCC**

As mentioned above, many cancer types have been linked to aberrant immune responses [8, 13, 16, 17]. One such cancer is HCC, which is one of the most common and aggressive human malignancies worldwide [18]. Patients with HCC usually survive for a period of less than 1 year after diagnosis. Although surgery remains the most effective approach to intervene in HCC, a majority of HCC patients is still ineligible for surgical intervention [19]. For those who do qualify for surgery, the consequent improvement in long-term survival is only modest, as a result of the high rate of recurrence or intrahepatic metastases that develops from portal vein invasion or spread to other parts of the liver [20, 21]. To improve HCC prognosis and treatment, information about the phenotypic and molecular changes associated with the development of this disease must be determined.

Overall, several lines of evidence indicate that chronic infection with hepatitis viruses, hepatitis B virus (HBV) and HCV, are major risk factors for HCC development [22]. A survey of published clinical studies has shown that greater than 85% of HCCs worldwide retain markers for HBV and/or HCV [23–33]. The genome of HBV, a small, enveloped DNA virus, consists of four overlapping open reading frames, which encode seven different peptides including three HBV surface antigens (HBsAg); one HBV core antigen, and one X region, which encodes a viral transactivator (HBx), required for viral replication [34–38]. HCV, conversely, is a positive, single-stranded RNA molecule, which encodes a 3000-amino acid polyprotein, processed by viral and cellular components to produce at least 10 structural (E1, E2, and p21core) and nonstructural proteins (NS2, 3, 4A, 4B, 5A, and 5B) [26, 39, 40]. Coinfection with both viruses can be found in patients with HCC, and synergistic effects have been suggested in studies examining patients infected with both viruses [41–44]. The host immune responses to hepatitis viruses are fairly weak and fail to completely down-regulate or clear the infection, resulting in chronic stimulation of the antigen-specific immune response in persistently infected patients. The continued expression of cytokines and recruitment of activated lymphomononuclear cells to the liver affects many cellular pathways and ultimately results in fibrosis, cirrhosis, and/or HCC. Long-standing, inflammatory injury is therefore an important procarcinogenic factor.

In the liver, an organ enriched with immune cells, cell damage, and regeneration, mediated by viral hepatitis-induced immune responses, may lead to liver cancer by promoting cell proliferation and death [45–47]. It has been proposed that necrosis of hepatocytes, as a result of chronic inflammation and consequent regeneration, enhances mutagenesis in host cells, which can accumulate and culminate in HCC [48–50]. In fact, several lines of evidence indicate that the recruitment of inflammatory cells contributes to the secretion of cytokines and chemokines and lysis of infected cells, which causes much of the liver injury characteristic of acute HBV [47, 48, 50]. Upon recognition of target cells, NK cells may directly lyse HBV-infected cells and/or down-regulate HBV replication by producing IFN-γ and TNF-α [51]. IFN-γ also activates macrophages and increases TNF-α-mediated liver damage [52]. It has been shown that constitutive expression of IFN-γ in the liver causes chronic, active hepatitis by recruitment of lymphomononuclear cells, even in the absence of any viral infection [53]. The cytotoxic T cells (CD8+T) recognize viral peptides derived from phagocytosed and proteolytically cleaved HBV proteins, activate and differentiate B cells, and secrete IFN-γ and TNF-α, which inhibit the replication and gene expression of HBV. In a study of HBV-transgenic mice, antigen, nonspecific-inflammatory cells of the host were recruited to the liver after injection with HBs-specific CD8+ T
cells and formed necroinflammatory foci associated with much of the histopathologic manifestations of liver disease [54]. In a separate study, chronic HBV-specific, T cell-mediated liver disease was sufficient to induce HCC in HBV in vivo models [55].

The HCV-mediated cellular immune response is weak, and a HCV-neutralizing antibody, which can protect against HCV infection has not yet been detected [56]. The immune reactivity observed in the liver of HCV-infected patients is largely un-specific, and innate cells are thought to play a major role in HCV immunopathology [46, 49]. Intrahepatic production of cytokines and chemokines by HCV infection promotes the recruitment of nonspecific lymphocytes. In the absence of viral clearance, this pathway boosts itself, leading to necroinflammatory and fibrotic liver disease. In HCV p21core-transgenic mice, a significant lymphocyte infiltration was observed in the portal tracts of livers and was associated with increasing serum alanine transaminase (ALT) levels [57]. These changes in liver function are associated with the development and progression of HCC.

In addition, hepatic viruses have been able to mechanistically evade the immune system and persist in the host through their ability to rapidly mutate under immune pressure, ultimately resulting in liver injury [58–61]. Some mutations create antagonistic peptides with the potential to inactivate T cells that are specific for the prototype HCV antigenic epitope and allow for viral escape. HBV variations within T cell epitopes have been demonstrated in chronic HBV [62, 63]. These variant sequences may down-regulate MHC class II genes in B cells and prevent B cell apoptosis [74]. The envelope protein, HCV E2, has been shown to bind CD81 on NK cells and inhibit NK cell function [75]. In addition, the nonstructural HCV phosphoprotein, NS5A, is implicated in mediating HCV resistance to IFN-α through its ability to inhibit IFN-α-induced protein kinase [76]. Similarly, HBx, the smallest protein encoded by HBV, has been shown to interact directly with and modulate the function of mediators of the inflammatory process including IL-8, ICAM-1, and the MHC factor. HBx also activates two transcription factors, NF-κB and NFAT, implicated in the regulation of cytokine expression (IL-6, IL-8, and TNF-α) [77]. Thus, hepatitis virus-encoded proteins are able to directly alter cytokine expression to modulate the immune response in the liver and thus, may contribute to HCC.

Inflammatory responses to viral infection are followed by tissue repair and liver regeneration, processes that involve cell proliferation. In the liver, accumulation of mutations caused by aberrant cell proliferation can increase the transformation potential of hepatocytes. Another consequence of chronic inflammatory responses initiated by viral infection is the release of free radicals, such as reactive oxygen species and NO reactive species [13]. These oxyradical species released at the sites of inflammation by recruited leukocytes can induce several alterations including structure/function modifications of cancer-related proteins and gene mutations, including those related to cell-cycle control, apoptosis, lipid peroxidation, and DNA repair damage, and thus, free radicals have been associated with carcinogenesis. Hovhannessian et al. [78] have shown that p53 is post-translationally modified at critical residues after exposure to NO and its derivatives. Furthermore, it was shown that nucleotide transversions at codon 249 of p53 occur with higher frequency in cases of oxyradical overload diseases [79]. In addition, accumulation of DNA adducts formed by oxidative stress in HBV-transgenic mouse have been correlated with HCC progression [80]. Thus, host-viral interactions may result in unwanted pathological changes, which in turn favor neoplastic transformation. Since the discovery of HBV and HCV, many advances have been made in understanding the molecular mechanism and treatment of individuals infected with these viruses. Nevertheless, it remains controversial as to whether they play a direct or merely indirect role in the pathogenesis of HCC.

**CYTOKINES AND HCC**

Mounting evidence indicates the involvement of cytokines in hepatocarcinogenesis. Thus, various avenues have been taken to elucidate changes in cytokine expression levels in patients with HCC. The mRNA and protein expression of cytokines in HCC and liver-related diseases has been demonstrated by immunohistochemistry (IHC), quantitative real-time PCR (qRT-PCR), and ELISA. To determine whether cytokine expression correlates with disease progression, many comparisons have been made, including HCC tumor and nontumor samples, chronic liver disease (CLD) patients and normal, healthy
individuals, and cytokine measurements in response to various treatment regimens. As cytokines are secreted and can be measured in the serum and plasma, several groups have analyzed certain cytokines for their predictive capacity (Table 1).

**Cytokine expression levels in HCC tumors versus nontumors**

To assay immune response changes in HCC patients, the expression levels of pro- and anti-inflammatory cytokines have been quantified in HCC tumors and comparative normal samples. Of the many anti-inflammatory cytokines, the most studied with regard to HCC expression is IL-10. Several groups have shown that IL-10 is highly expressed in HCC tumors and individuals with HCC versus nontumorous-surrounding tissue controls or healthy cohorts, respectively [81, 93, 99, 100, 107]. These studies suggest that increases in IL-10 and perhaps other Th2 cytokines correlate with progression. Further studies are thus warranted to examine the expression levels of other Th2-related cytokines in liver-related disease.

Conversely, many studies have shown associations between the expression of several Th1 cytokines and HCC. Proinflammatory IL-1β was shown to be elevated in HCC patients compared with healthy individuals [82]. However, by qRT-PCR analysis, IL-1β mRNA was lower in tumors versus the tissue microenvironment [83]. In studies by Kakumu et al. [99], serum IL-15 was higher in HCC and reflected the degree of inflammation in the liver. The expression of IL-18 was measured by IHC and shown to be slightly higher in normal surrounding tissue versus the tumor [93]. TNF-α expression was elevated in HCC patients, especially those with recurrence [82, 84]. In addition, the levels of the TNF-αRs, TNFαRI and TNFαRII, were higher in HCC patients when compared with healthy individuals [99]. However, in other studies, TNF-α levels were lower in HCC tumor tissue versus the tissue surrounding the tumor and in HCC patients versus healthy individuals [83, 100]. In situ hybridization studies revealed that tumor and nontumor sections were more positive for IFN-α mRNA than histologically normal liver; however, deficient IFN-α expression in HCC was shown at the protein level [94]. The expression of IFN-γ was measured in HCC by IHC and shown to be slightly higher in normal, surrounding tissue [93]. In another study, IFN-γ was not detected in HCC, and the authors concluded that IFN-γ may not play a large role in liver inflammation [95]. The proinflammatory cytokines IL-12 and IL-2 are also increased in HCC [81, 90]. Thus, Th1 cytokines are mainly up-regulated in HCC.

**Table 1. Selected Cytokines Associated with HCC**

| Gene symbol | Function | Cell source | Disease association | HCC-associated polymorphism | Tumor expression | Prognostic | Ref # |
|-------------|----------|-------------|---------------------|-----------------------------|------------------|-----------|------|
| IL-1B       | Activates T and B cells and monocytes | T or B cells, monocytes, macrophages | HBV, HCV, HCC | yes | up | ? | [81–89] |
| IL-2        | Growth and differentiation of all immune cells | T cells | HBV | ? | ? | yes | [90–92] |
| IL-12B      | Stimulates T cells, induce IFN-γ, defense against pathogens | B cells, monocytes, macrophages | HBV, HCC | yes | up | ? | [81, 85, 86] |
| IFNG        | Monocyte activator; regulate immune and inflammatory responses | T cells, macrophages, NK cells | HBV, HCV, HCC | yes | up, down | yes | [93–98] |
| TNFA        | Mediator of inflammatory and immune functions | T or B cells, monocytes, macrophages | HBV, HCV, HCC | yes | up, down | yes | [82–84, 86, 96, 99–106] |
| IL-4        | Induces secretion of Ig by B cells; pleiotropic effect on T cells | T or mast cells | HCC | yes | ? | ? | [98] |
| IL-10       | Blocks Th1 T cell cytokines; stimulates proliferation of B cells, thymocytes, and mast cells; stimulates IgA production by B cells | T or B cells, monocytes | HBV, HCV, HCC | yes | up | yes | [81, 85, 92, 93, 96–100, 104, 107–109] |
| IL-6        | Stimulates thymocyte proliferation, B cell maturation and proliferation, and fibroblast growth factor activity | Macrophages | HBV, HCC | yes | up | ? | [82, 85, 96, 97, 108, 110–116] |
| TGFB        | Suppresses T cell growth, differentiation | T cells, platelets | HBV, HCV, HCC | yes | up, down | yes | [96, 97, 117–122] |
Several cytokines, which do not function specifically in a Th1- or Th2-restricted group or do not fall into the IL family, have also been correlated with HCC. IL-6, a cytokine categorized as pro- and anti-inflammatory with a multifunctional role in host defense, was present at higher levels in HCC patients compared with healthy individuals [110]. The glycoprotein TGF-β was demonstrated to have lower or similar concentrations in HCC and the surrounding tissue [117, 118]. However, in other studies, TGF-β was higher in HCC patients versus healthy individuals [119, 120]. The level of macrophage migration inhibitory factor (MIF), a proinflammatory and carcinogenic cytokine, was shown to be higher in sera from patients with HCC than in healthy individuals [123]. Similarly, the levels of hepatocyte growth factor and c-reactive protein in HCC were higher compared with healthy individuals [110]. A study of the macrophage-related cytokine M-CSF showed that an increase of this cytokine correlated with hepatic inflammation and necrosis [101]. These studies therefore show that the expression of cytokines is generally altered in HCC.

Although a multitude of studies have presented evidence for changes in cytokine expression in HCC tumors, the direction of the change is not always consistent. In addition, the cytokine levels found in the local regions, such as near or within the tumor, do not necessarily correspond to their level in serum. This may be a reflection of the differences in the particular samples examined or the complex nature of the immune network in response to malignant changes or the activity of viral hepatitis. In addition, these approaches assay cytokine levels in a “snapshot-type” manner, which may not reflect the actual directionality of the response. Conclusive findings will require additional studies and an improvement in the technologies available for measuring cytokine levels.

**Cytokine expression levels and chronic liver disease**

It has been shown that ineffective cytokine responses are one of the reasons for the failure to suppress hepatitis viral replication. Such responses do not eliminate the virus and therefore allow chronic disease states to form. To understand the changes that occur in immune response to chronic infection, cytokine levels have been measured in CLD patients infected with HBV and/or HCV. Similar to HCC, the most studied anti-inflammatory cytokine with regard to CLD is IL-10. Song et al. [85] measured the serum level of IL-10 and found that it was uniformly low and not associated with clinical presentation. However, in a study by Kitaoka et al. [81], IL-10 was associated with the progression of CLD type C. Therefore, increases in IL-10 may be associated with CLD; however, additional studies of this cytokine and other anti-inflammatory cytokines need to be performed to determine their role in CLD.

Conversely, several studies have shown that proinflammatory cytokines are associated with CLD. Higher levels of the proinflammatory cytokines IL-12 and IL-18 are associated with CLD, predominantly in patients with liver cirrhosis, chronic hepatitis, and HBV positivity [81, 85, 86]. IL-1β, in particular, was elevated in acute and chronic HCV patients compared with healthy individuals [82]. The level of TNF-α was found to be higher in patients with cirrhosis or acute/chronic hepatitis when compared with healthy patients [82, 86, 102]. An increased level of TNF-α was also shown to correlate with hepatic inflammation, necrosis, and hepatic failure [86, 101]. The levels of TNFαRI and TNFαRII were also shown to be increased in the livers of CLD patients [99]. However, in a HCV+ cohort studied by Zekri et al. [100], although TNF-αRs were higher in CLD patients and healthy individuals, the level of TNF-α was lower. In liver biopsies of HCV patients, acute and chronic samples were positive for IFN-γ, and IL-2 was undetectable, despite positivity for the IL-2 receptor [91]. The expression of IFN-γ was associated with chronic HCV and similar to proinflammatory IL-1α, was up-regulated in chronic hepatitis and cirrhotic tissue versus normal patients [93, 102]. However, in another study, IFN-γ was not detected in HCC patients chronically infected with HBV, and the authors concluded that IFN-γ may not play a large role in liver inflammation [95]. Therefore, cytokines may have altered expression in cirrhotic disease, dependent on HBV or HCV status.

Other non-Th1/Th2 cytokines have also been measured in CLD. IL-6 has been associated with HBV-infected patients, and higher expression levels were observed in liver cirrhosis versus healthy individuals [85]. Elevated serum IL-6 levels have also been observed in HCV patients and were associated with the presence of cirrhosis [111]. In a separate study, IL-6 was elevated in acute HCV and cirrhosis patients compared with healthy individuals [82]. However, IL-6 has also been shown to be down-regulated in chronic hepatitis and cirrhotic tissue versus normal liver tissue [102]. Okumoto et al. [121] studied the relationship of plasma TGF-β to antitumor immunity and found higher levels of TGF-β in HCC patients versus healthy individuals. Similarly, TGF-β serum level was higher in patients with alcohol-related cirrhosis and chronic HCV patients versus healthy individuals [119, 120]. Therefore, TGF-β may be associated with malignant transformation or progression of chronic disease. The level of MIF was shown to be higher in sera from liver cirrhosis patients versus healthy individuals, and hepatocytes from patients with liver cirrhosis expressed high levels of MIF [123]. In addition, M-CSF cytokine production was greater in cirrhotic versus noncirrhotic patients in a study by Itoh et al. [101]. Taken together, pro-and anti-inflammatory cytokines can deviate from normal immune responses and elicit changes that contribute to chronic infection.

**HCC cytokine expression level changes in response to treatment**

Several studies have demonstrated that changes in cytokine expression result from various treatment regimens for HCC. In this vein, many studies have analyzed cytokine levels in patients before and after resection. For example, when compared with healthy individuals, HCC patients had higher preoperative levels of IL-6, which rose dramatically after resection. IL-6 has also been shown to increase significantly after hepatectomy, suggesting that hepatic resection is followed by the enhanced production of cytokines [112]. Postoperative IL-6 levels in patients with HCC resection was also tested by Chau and colleagues [108]. In this study however, the level of IL-6 decreased following tumor resection. Furthermore, in a study of 139 HCC patients with hepatic resection, IL-6 levels were not consistent with the occurrence of postoperative complications.
IL-1β has also been measured in HCC undergoing resection [86]. Higher IL-1β was found in cirrhotic patients when compared with healthy individuals or preoperative patients and increased postoperatively. Preoperative cytokine production was greater in cirrhotic versus noncirrhotic patients, and cytokine productivity increased after resection. In Sato et al. [86], TNF-α was measured in HCC patients undergoing resection. Higher TNF-α was found in cirrhotic patients when compared with healthy individuals and preoperative patients. The level of this cytokine increased postoperatively and correlated with postoperative, hepatic failure. In another study, G-CSF was measured in LPS-induced macrophages from PBMC isolated from seven HCC patients undergoing resection [101]. Preoperative cytokine production was greater in cirrhotic versus noncirrhotic patients, and cytokine productivity increased after resection. In sum, cytokine levels appear to increase following hepatic resection and may play protective and repair roles as a result of surgically induced liver injury.

IFN-α treatment has been shown to affect viral load in hepatitis-infected patients. Many studies have therefore measured cytokine levels in patients undergoing IFN-α treatment. Kitaoka et al. [81] assayed the levels of IL-10 and IL-12 in association with the progression of CLD type C and in response to IFN-α therapy. In this study, IL-12 and its receptor were highly expressed in HCC, followed by cirrhosis and chronic hepatitis, and were significantly elevated after IFN-α treatment, and no changes in IL-10 expression were observed. In studies by Kakumu et al. [99] of healthy individuals or patients with CLD and HCC, it was found that the serum levels of IL-10 and IL-15 correlate with disease progression and may reflect the degree of inflammation in the liver. HCC patients had the highest values for IL-10 and IL-15, and IFN-α treatment was shown to suppress these levels. In this study, the authors also showed that both types of TNF-αRs correlated with disease progression, and IFN-α treatment did not affect their level. Therefore, IFN-α treatment appears to affect the levels of pro- and anti-inflammatory cytokines. Although there has been limited success with IFN-α treatment, many patients do not respond to this therapy [124, 125]. In our laboratory, we are currently testing the ability of certain cytokines to discriminate HCC patients who are most likely to benefit from IFN-α treatment.

Cytokine levels have also been measured in patients undergoing other treatments for liver-related disease. In a study of 26 HCC patients with cirrhosis ± splenectomy, no differences were observed in IL-10 and IL-2 between the groups before surgery [92]. However, after surgery, IL-10 decreased in patients with splenectomy. In addition, IL-1β was measured in 43 HCC with transarterial chemoembolization (TACE) treatment [101]. LPS-induced production of IL-1β in PBMC increased after TACE and correlated with hepatic inflammation and necrosis. In addition, M-CSF was also measured in 43 HCC patients undergoing TACE treatment. Serum M-CSF increased after TACE and correlated with hepatic inflammation and necrosis. In sum, cytokine levels are altered after various treatment regimens for liver-related disease; however, the direction of the change is not always consistent between studies. This may be a reflection of the particular cohorts studied and must be reconciled to elucidate the positive, cytokine-based reaction of certain patients to liver disease-related treatment.

HCC cytokine expression levels and metastasis/recurrence

Some studies have correlated changes in cytokine expression with HCC metastasis and/or recurrence. In a rat model, IL-6 has been implicated in HCC metastasis, as highly metastatic HCC (metastatic to the abdominal cavity) has been shown to release more IL-6 in serum [114]. Exogenous addition of IL-6 did not affect primary tumor formation but did affect the metastatic potential of tumor cells when compared with tumor cells expressing endogenous IL-6. Therefore, IL-6 production could be used to identify cells with metastatic potential and did not appear to be essential for primary tumor establishment. Furthermore, Coskun et al. [115] showed that in breast cancer patients, higher serum levels of IL-6 could be used to distinguish primary or metastatic liver tumors from benign HCC lesions. The proinflammatory cytokines TNF-α, IL-1α, and IL-1β were measured in HCC patients before and after operation or in patients with metastatic liver carcinoma [84]. TNF-α and IL-1β were high in HCC and metastases to the liver before operation compared with healthy individuals and increased postoperatively. In another study, higher levels of IL-1β and TNF-α were found in the tissue surrounding HCC and hepatic metastases than in the tumor [83]. High expression of IL-8, an anti-inflammatory cytokine with angiogenic action, in cancerous liver tissue was associated with a higher frequency of portal vein, venous, and bile duct invasion in HCC patients with surgical resection and may therefore be important in invasion and metastasis [126]. High MIF levels, another cytokine involved in angiogenesis, have been associated with frequent intrahepatic recurrence in HCC patients [127]. A study by Wang et al. [128] was conducted to elucidate the mechanism of IFN-α on the inhibition of metastasis and recurrence of HCC. Nude mice with HCC xenografts with high metastatic potential (intrahepatic and lung) underwent resection and were then s.c.-treated with IFN-α at different doses. IHC analysis revealed that IFN-α mediated antiangiogenesis by down-regulating vascular endothelial growth factor (VEGF) but had no effect on fibroblast growth factor. In our laboratory, we have conducted a study that will be discussed later in this review, which demonstrates that pro- and anti-inflammatory cytokine imbalances occur in HCC patients with venous metastasis. Thus, it appears that some cytokines may contribute to the metastatic process, and several play a role in the prometastatic angiogenic phenotype.

HCC cytokine expression levels and prognosis

As cytokines are expressed in the serum and plasma, they are readily suitable for use in clinical settings as prognosticators (Table 1). Thus, the ability of pro- and anti-inflammatory cytokine levels to predict patient outcome has been studied. The clinical significance of postoperative, anti-inflammatory IL-10 levels in patients with HCC resection was tested by Chau et al. [108]. IL-10 levels were significantly higher in HCC than in healthy individuals. Patients with high IL-10 had a worse disease-free survival, and a multivariate analysis implied that
IL-10 may be a predictor of the postresection outcome of HCC patients. The proinflammatory cytokine IL-2 was measured by qRT-PCR and compared with the density of CD8+ tumor-infiltrating lymphocytes (TILs) detected by IHC in 59 HCC resection patients [90]. A decrease in CD8+ cells was seen in tumor versus noncancerous tissue, and an increase in CD8+ cells was observed in tumors with IL-2 expression. As patients with IL-2+ tumors had a favorable prognosis, the authors concluded that IL-2 activates CD8+ T cells produced by TILs and may be a good prognostic marker. Thus, changes in pro- or anti-inflammatory cytokines in HCC may be used to screen individuals for potential disease outcome.

The prognostic association of other non-Th1/Th2 cytokines has also been tested in HCC patients. The clinical significance of postoperative IL-6 levels in patients with HCC resection was tested by Chau and colleagues [108]. IL-6 was significantly higher in HCC than in healthy individuals; however, IL-6 did not correlate with patient survival in this study. Okumoto et al. [121] studied the relationship of plasma TGF-β to antitumor immunity and prognosis in 70 patients with unresectable HCC, age-matched, healthy individuals, and 32 cirrhosis patients. HCC patients with high TGF-β concentration had a shorter survival period than those with concentrations below that of healthy individuals. Therefore, the concentration of TGF-β was shown to be a predictor of outcome of patients with unresectable HCC. In other studies, patients with high MIF had a worse survival outcome than patients with low MIF [127]. Another significant predictor of poor outcome is IL-18, which is up-regulated in HCV-positive patients [129]. In summary, these studies show that the expression level of several cytokines is associated with disease outcome, and they may therefore serve as HCC prognosticators. However, these findings should be tested in independent datasets to assure validity. In addition, quick and cost-effective diagnostic kits will need to be available for optimal clinical use of the cytokines that are demonstrated to be HCC prognostic markers.

Cytokine polymorphisms and HCC

The alteration of cytokine expression levels and the functional consequences of these changes in HCC may be caused by the response of the immune system to the presence of a primary lesion. However, an individual’s genetic constitution may also play an important role in affecting elements of the immune system and generating tumorigenic effects. Many studies have been conducted to analyze single nucleotide polymorphisms (SNPs) as genetic markers as a result of their high density and even distribution in the human genome. As such, many groups have used SNPs for fine-mapping disease loci and for analyzing the association of genes with disease outcome to determine how or whether inherited germ-line mutations can contribute to the susceptibility of certain individuals to cancer development. In this line, several studies have identified cytokine SNPs, which are functionally associated with liver disease and/or HCC (Table 1).

High levels of proinflammatory TNF-α have been associated with carcinogenesis. In particular, the TNF-α (−308) SNP in the promoter region of the gene, which includes TNF-α1 (−308G) and TNF-α2 (−308A) alleles, is associated with cancer susceptibility and induced expression of TNF-α. In a case study, 74 HCC and 289 healthy individuals were assayed for their −308 genotype [103]. Carriage of the TNF-α2 allele was associated with an increased risk (3.5 odds ratio) of HCC, and a significant increased risk of death was associated with TNF-α1/TNF-α1, TNF-α1/TNF-α2, to TNF-α2/TNF-α2 genotypes. The TNF-α2 allele was therefore a significant predictor of HCC. In an analysis by Heneghan et al. [104], genomic DNA was analyzed from 98 HCC from a Chinese cohort and 77 family controls, 97 Hong Kong controls, and 96 European controls, who were healthy individuals. The TNF-α2 allele was associated with high TNF-α production in vitro and in vivo and found in 10% of the Chinese and 33% of the European subjects. Although no specific associations were found between the polymorphisms tested in this study, they concluded that the distribution of alleles may have accounted for the susceptibility of the Chinese population to develop HCC. The TNF-α genes are located in the central MHC complex. It has been shown that the region −323 to −285 encompassing −308 in the TNF-α2 allele binds NFs differently than TNF-α1 [105]. In this study, a G/A polymorphism allowed for NF-binding to TNF-α2 but not to TNF-α1, and TNF-α2 had greater transcription than TNF-α1. Therefore, the G/A polymorphism may play a role in altered TNF-α gene expression in patients with particular MHC haplotypes. Another study tested the role of the TNF-α polymorphism on clearance of HBV and outcome of HBV-related chronic hepatitis [106]. An Italian population of 184 chronic HBV carriers, who were HBsAg+, including HCC cases and 96 healthy individuals, was assayed. The TNF-α1 allele was found to be more frequent in the chronic carrier group in patients with a family history of HBV infection. The G/G genotype at −308 was found in all carriers with decompensated cirrhosis but only in 78% of healthy individuals. The distribution in the carrier group was not significantly different from the healthy group. SNPs at −1038/−863 and −863/−238 were in linkage disequilibrium. The TCGG haplotype (−1031, −863, −G308, −G238) was significantly associated with end-stage liver disease. The authors concluded that the TNF-α promoter polymorphism at −308G/G and the haplotype TCGG are associated with unfavorable prognosis in patients with chronic HBV infection. A Japanese cohort was used to determine the frequency of genotypes associated with cytokine polymorphism and their association with risk of HCC in HBV carriers [96]. No statistically significant differences in genetic polymorphisms of TNF-α were found. TNF-α polymorphisms were also not associated with HCC in a study of Japanese patients or in a study of 77 patients with chronic HBV examining TNF-α (−308) [97, 130]. Although the data regarding TNF-α association with disease outcome are not straightforward, perhaps as a result of analyses in differing ethnic cohorts, a relationship between increased levels of this cytokine and HCC has been observed.

Several polymorphisms of proinflammatory IL-1β have been reported, and the IL-1B-31 genotype and IL-1RN have been associated with enhanced IL-1β production. The IL-1B-31 genotype T/T or IL-1B-511/-31 haplotype C/T was examined in a study of 274 Japanese patients with HCV + HCC and 55 healthy individuals [87]. These polymorphisms were found to be associated with the presence of HCV + HCC. Another study investigated an IL-1β polymorphism in 364 Japanese
HCV patients, 146 of which had HCC [88]. The IL-1β-511 genotype T/T was a significant risk factor for HCC. The C-511T-IL-1β polymorphism was also examined in 136 Thai patients with chronic HBV. In this cohort, the IL-1β-511 genotype C/C was found to be significantly higher in patients with HCC versus healthy individuals. The IL-1β polymorphism may therefore be a genetic marker for the development of hepatitis-related HCC [89].

Proinflammatory IFN-γ polymorphisms have also been associated with HCC. In a study of 77 patients with chronic HBV, 23 low and 23 high HBV replicative carriers, 21 liver transplant candidates and 10 HCC, a high statistical difference in the distribution of the IFN-γ polymorphism (+874) was shown between chronic HBV and healthy individuals [97]. The majority of the patients produced less IFN-γ than in healthy individuals, and no difference in IFN-γ production was seen in patients with low versus high levels of HBV replication. IFN-γ (+874), a low-expressing IFN-γ polymorphism, was assayed in Japanese HCC patients with HBV infection; however, no difference was observed compared with those patients without HCC [96]. In a Chinese cohort of 250 HCC and 250 hospital controls (healthy individuals), IFN-γ + 847 T/A was associated with a nonsignificant increase in HCC risk [98]. IFN-γ + 847 T/T homozygosity was associated with elevated IFN-γ production, and IFN-γ + 847 A/A was associated with reduced IFN-γ production. Furthermore, the risk increased with increasing the number of Th1 genotypes including IL-12 and IL-18. Thus, IFN-γ polymorphisms in general may lead to a decrease in IFN-γ activity and corresponding increase in HCC risk.

The association of Th2 cytokine SNPs and the risk for developing HCC are less clear. A polymorphism of IL-10 in 1078 chronic HBV patients and HCC (IL-10-h12 haplotype) was strongly associated with HCC and with an increased production of IL-10 [109]. The frequency of susceptibility increased in order from chronic hepatitis to liver cirrhosis and HCC among HBV patients. A survival analysis of the data revealed that the onset age of HCC accelerated in chronic HBV patients who carried this haplotype. In a Chinese cohort, IL-4–589C/T and IL-10 (–1082G/A, –819C/T) were associated with a nonsignificant reduced risk of HCC, and the risk decreased with increasing the number of Th2 genotypes [98]. IL-4–589C/T was associated with increased IL-4 production, and IL-10 polymorphisms (–1082G/A, –819C/T) were associated with reduced plasma IL-10 levels. In a study of 77 patients with chronic HBV and a separate study of 236 Japanese patients with HBV infection, no genetic difference was observed in TGF-β (–509T/T and C/C) have been associated with increased IL-6 levels and were genotyped in chronic HBV patients [116]. IL-6–597G>A and –174G>C had significant frequency in the Korean population, but no significant associations were found between IL-6 variants and disease outcome in these patients. Genetic differences in IL-6 (–592) were not found in two other separate studies of patients with chronic HBV (–592) [96, 97]. Further studies are therefore warranted to clarify the differing findings with regard to IL-6 and HCC risk.

In sum, many cytokine polymorphisms have been associated with an increased risk of HCC. These findings suggest that genetic alterations, which lead to changes in Th1- and Th2-related cytokine levels, can influence an individual’s disease outcome. However, a clear-cut association between a particular cytokine SNP and HCC has yet to be determined. Observations have differed between studies regarding HCC risk and a particular cytokine polymorphism, perhaps as a result of the differences in specific polymorphisms or populations assayed. A large and comprehensive population-based SNP study may help to clarify these results.

Cytokines and the mechanism of HCC

Although many studies have examined cytokine expression changes in HCC, numerous investigators have focused their efforts on studying the functional consequences that correlate these changes with disease outcome (Table 1). The proinflammatory cytokine IFN-α is currently used for treatment of chronic viral hepatitis and to prevent development of HCC. IFN-α can transcriptionally regulate MHC Class I and Fas through interaction with IFN-αR. It has been shown that normal hepatocytes express weak levels of IFN-αR, but the expression of IFN-αR is up-regulated in acute and CLD [131]. Further examination revealed that tumor size, ALT, metastasis, and proliferation were higher in IFN-αR-negative liver tissue cases. Therefore, the loss of IFN-αR might contribute to im-
mune escape. The IFN-α signaling pathway has also been studied using hepatoma cell lines: Hep3B, HepG2, Huh7, SKHep1, and Chang-Liver, to assay IFN-α binding to regulatory elements and analyze its effect on proliferation and apoptosis [132]. The IFN-α signaling pathway was functional in several cell lines, where a moderate, anti-proliferative effect, but not an apoptotic effect, was observed. IFN-α may therefore exert its effect through action on specific nuclear sequences that protect liver cells from transformation. Yamaji et al. [133] investigated the effect of IFN-α on the expression of another proinflammatory cytokine IL-15 in HCC cell lines and patients with chronic HCV and found that IFN-α up-regulated DC production of IL-15, which is capable of activating NK and CTLs. Therefore, IFN-α may function to control tumorigenicity, but alterations in its pathway perhaps caused by the presence of a tumor may shift its action toward a proangiogenic phenotype.

Proinflammatory TNF-α is produced in response to tissue injury and is associated with an increase in cell-cycle progression and oxidative stress through the formation of 8-oxo-deoxyguanosine, an established marker of DNA damage associated with chronic hepatitis in human livers [134]. To examine the cytotoxic role of TNF-α, TILs, isolated from 22 primary and metastatic liver tumors, were expanded in the combined presence of IL-2 and TNF-α, IL-2, and IL-4 or IL-2 alone [135]. TNF-α and IL-2 were the most effective in promoting outgrowth of CD3+CD8+ T cells and could induce cytotoxicity, which was further enhanced by IFN-α. Cytokine stimulation of TNF-α, IL-1β, or IL-18 has also been shown to induce expression of TRAIL in HCC cell lines (HepG2, Hep3B, Huh7) [136]. The expression of TRAIL on the HCC cell surface might contribute to tumor cell immune evasion by inducing apoptosis of activated human lymphocytes. An investigation of cytokine-induced tumor growth modulation during HCC progression in SV40 large T tumor antigen-transgenic mice revealed that an increased rate of liver growth correlated with an increase in TGF-β mRNA expression, which was particularly detected in early tumor development [137]. However, alterations of IL-1α, IL-1β, IL-6, IL-2, IL-4, or IFN-γ mRNA were not observed in this study. Therefore, TNF-α may inhibit carcinogenesis through cytotoxic induction; however, under certain circumstances, this cytokine can promote this process.

It has also been demonstrated that anti-inflammatory cytokines with angiogenic function, such as IL-8, can accelerate the proliferation of various HCC cell lines [126]. In a HCC mouse model, high angiogenic activity was associated with attenuated lymphocyte extravasation and correlated with the expression of anti-inflammatory IL-10 [138]. IL-22, an IL-10-related cytokine released by T cells, is normally highly expressed in hepatocytes. Blocking IL-22 was shown to reduce STAT3 and worsen liver injury, and injection with recombinant IL-22 attenuated injury. Overexpression of IL-22 in HepG2 promoted cell growth and survival and activated STAT3 to induce antiapoptosis (Bcl-2, Bcl-xl, Mcl-1) and pro-mitogenic proteins (myc, cyclin D1, Rb2, CDK4) [139]. Therefore, T cell activation by anti-inflammatory cytokines may play a role in prevention and repair of liver injury as well as promoting this process by differential signaling from proangiogenic cytokines.

The mechanisms leading to HCC by non-Th1/Th2 cytokines have also been studied. Chabivolsky et al. [140] found that TGF-β stimulated apoptosis and mitosis of hepatocytes in a chemically induced mouse model of liver carcinogenesis. In a study by Murata et al. [141], TGF-β suppressed viral RNA replication and protein expression from the HCV replicon and was associated with cellular growth arrest, which was dependent on Smad but not MAPK. In addition, Activin A, a member of the TGF-β family, and its receptors are expressed in HCC, and have been shown to stimulate VEGF transcription, DNA binding, and the transactivation potential of specificity protein 1 [142]. Therefore, TGF-β may function to enhance tumorigenicity. In other studies, patients with high MIF levels have high α-fetoprotein (AFP) levels, which are associated with hypoxic stress and angiogenesis [127]. In a separate study, IL-6, IL-6R, and downstream pathway members were measured in liver samples from a HCC rat model versus normal surrounding tissue [143]. HCC had decreased IL-6R compared with normal surrounding tissue, and increased IL-6 mRNA levels led to an increase in STAT and ERK. IL-6 was also shown to inhibit serum-stimulated DNA synthesis and cell mitogenesis in HCC cells in vitro. Therefore, IL-6 and other non-Th1/Th2 cytokines may contribute to the pathogenesis of HCC at the stages of transformation and metastasis.

As hepatitis viruses are major risk factors for HCC, cytokine interactions with these viruses have been documented. In HCV studies, a decreased, therapeutic response to IFN-α has been shown in chronic HCV patients. To analyze the mechanism behind this observation, HepG2 cells were treated with IFN-α ± TNF-α to assay NF-kb modulation [144]. IFN-α and alcohol induced NF-kb activation and augmented TNF-α-induced NF-kb binding in cells. Szabo et al. [144] also investigated the effect of IFN-α and alcohol on monocytes. IFN-α is produced by and affects monocyte populations, and alcohol has been shown to alter monocyte production and antigen-presenting activity. Szabo and colleagues [144] found that IFN-α treatment induced monocyte production of TNF-α, IL-6, and IL-12. Therefore, IFN-α can increase sensitivity to TNF-α and alcohol-mediated activation of inflammatory cytokines and could therefore be a key element in the antiviral response in chronic HCV. IL-18, a proinflammatory cytokine, was shown to be up-regulated in HCV-positive patients. In a cohort of 65 HCV + HCC with resection, IL-18 was expressed in all samples and in two HCC cell lines [129]. The expression of cytokines has also been evaluated in a HCV mouse model overexpressing p21<sup>core</sup> with associated HCC [145]. The authors observed increased mRNA and protein levels of TNF-α and IL-1β and enhanced activity of JNK and API. However, no effect was observed on Ikb and NF-kb. Alcohol is known to increase liver fibrosis and HCC in chronic HCV patients. When looking at the effect of chronic alcohol consumption in HCV core-expressing, transgenic mice, Perlemuter et al. [146] observed that hepatic expression of TGF-β and TNF-α increased. The expression of the HCV core in stably transfected T cells has also been shown to correlate with reduced IL-2 promoter activity and IL-2 production in response to TCR triggering [147]. IL-4, IL-10, IFN-γ, and TNF-α were increased moderately, and these alterations resulted in perturbed MAPK responses. In a study analyzing the effect of HCV NS5A
Cytokine-based HCC therapy

As the levels of many cytokines are altered in HCC, as evidenced by measurement in serum, qRT-PCR, and IHC, and as these changes can have profound pro- or antitumor effects, much emphasis has been placed on cytokine-based therapies, which can be categorized as antiviral or antitumor. Although many hurdles still remain, some of these avenues show therapeutic promise. A few of these studies are described below.

Antiviral strategies have been used to treat patients with HCC related to chronic HCV infection. Current evidence is sufficient to recommend IFN-α treatment for all patients with acute hepatitis. With the advent of a combined, conventional IFN-α and ribavirin therapy, the sustained, virological response rate has been improved greatly compared with IFN-α monotherapy. However, it is necessary to search for the underlying mechanisms for a better treatment outcome with IFN-α plus ribavirin combination therapy in patients. Whether the long-term effects of IFN-α plus ribavirin therapy can reduce the incidence of HCC needs to be established. The current gold standard of efficacy for treatment of patients with chronic HCV is the combination of pegylated IFN-α (PEG-IFN-α) and ribavirin, which is more effective in improving liver histology. PEG-IFN-α (12 KD) has a small, linear polyethylene glycol (PEG) moiety and has a longer half-life than conventional IFN-α, which allows for significant increases in sustained, virological response rates. Tolerability and compliance to therapy are still a problem, as 15–20% of patients within trials and >25% in clinical practice withdraw from therapy. In addition, there is much debate regarding the optimal dose and duration of PEG-IFN-α treatment, and the optimal dose of ribavirin has yet to be determined. Monotherapy with PEG-IFN-α induces a marked reduction in staging in virological-sustained responders and to a lesser degree, in patients who relapse but provides no benefit to nonresponders after 24–48 weeks of treatment. The use of maintenance therapy in virological nonresponders aiming to improve histology should be considered experimental and of unproven benefit. Pooling data from the literature suggests a slight preventive effect of IFN-α on HCC development in patients with HCV-related cirrhosis. Despite the low magnitude of this effect, some patients have benefited from such regimens.

Antiviral therapy has also been used for HCC patients infected with HBV. A recent study demonstrates a clear association between viral load and HCC risk [154]. IFN-α has been used effectively for anti-HBV therapy with a good, early response. However, a high frequency in relapse is a major problem for long-term use as a result of viral mutation and development of resistance. A future direction may be to identify better antiviral compounds that can be used together with or following IFN-α treatment. One such compound with beneficial potential may be lamivudine. Thus, antiviral therapies may help to delay the development of HCC.

Antitumor strategies for patients with HCC include various methodologies to boost or decrease the level of certain cytokines, particularly in the liver, or harness and expand reactive immune cells, which can be re-infused, and release their therapeutic cytokines. An example of the use of immune cells for immunotherapy is adoptive transfer. This method exploits the antibody response from a patient’s serum or from another patient (autologous or allogeneic), where tumor-reactive T cells (TILs) are isolated and expanded and then re-infused. However, only a fraction of the patients responds as a result of complications with retention of specificity and function, survival and migration to the tumor site, and graft-versus-host disease. In other types of regimens, immune cells are harnessed and expanded and then infused in the presence of cytokines to help to stimulate an inflammatory response. Local-regional immunechemotherapy with lymphokine-activated killer cells (LAK) have been used as a therapeutic approach for HCC. LAKs produce IL-2, IFN-γ, and IL-12 and thereby induce cytolytic activity [155]. DC pulsed with heat-shock protein 70 peptide have been shown to stimulate high T cell proliferation, cytokine secretion, and induction of CTLs, which specifically killed HCC cells by a MHC Class I-restricted
mechanism [156]. NKT cells have been proposed to mediate the effects of IL-12 [157]. Ex vivo immune-modulated NKT cells in BALB/c mice transplanted with human Hep3B HCC led to suppression of HCC [158]. These studies therefore provide an opportunity for the enrichment of cells with cytokine-producing ability and/or cytotoxicity in primary tumors and perhaps metastatic liver cancer.

Many therapy-based studies have also been performed to boost cytokine responses via different delivery mechanisms, and efforts have been made to deliver cytokines specifically to the liver. In Junbo et al. [159], therapeutic genes, including cytokines, were transferred into hepatoma cells via the hepatocyte receptor. An antitumor response of IL-12 was also tested in a gene-therapeutic HCC model using an adenoviral vector carrying IL-12 (Adv-IL-12), which caused regression and was associated with an immune infiltrate, potentially involved in the inhibition of angiogenesis [157]. Studies with Adv-IL-12 therapy of woodchucks with primary HCC induced by woodchuck hepatitis virus have shown substantial tumor regression and increased levels of CD1+ and CD8+ T cells and IFN-γ [160]. Intrahepatic delivery of IL-12 in BALB/c mice could inhibit tumor growth by allowing early infiltration of macrophages and lymphocytes [161]. Combined immunotherapy in an immunogenic HCC mouse model with IL-12 and GM-CSF revealed induction of a strong antitumor cellular response (better than each alone) and did not cause the severe side-effects associated with IL-12 alone [162]. Receptor-mediated gene delivery of IL-2 into HepG2 has been tested using EBV vectors, which allowed for specific binding of IL-2 to cell-surface receptors on the target cell and elevated levels of IL-2 gene expression [159]. In addition, a HSV amplicon carrying IL-2 in a rat HCC model significantly reduced tumor burden, suggesting that vascular regional delivery of HSV vectors could be used to induce antitumor efficacy [163]. Liver-specific albumin and tumor-specific AFP have been assayed for their ability to achieve IL-2/IFN-α2b HCC treatment. This method has led to growth suppression after retrovirus infection [164]. Use of Adv-AFP to direct IL-2 expression in a HCC-specific manner via intratumoral injection in established HCC xenografts growing in CB-17/SCID mice was shown to result in growth retardation and regression of established hepatomas [165]. Liver-directed IFN-γ gene therapy in a mouse model of chronic HBV infection has also been used [166]. Although methods of delivery remain to be improved, these studies are proof of principle and lead the way for future advances.

Cytokine-based treatments using other methodologies can also be found in the literature. Studies have been conducted with tumor vaccines using HCC cells/tissue fragments and biodegradable microparticles encapsulating GM-CSF and IL-2 along with adjuvant. This combination protected a large percentage of syngeneic mice from HCC cell challenge [167]. Oral immune regulation for HBV antigens (HBsAg, PreS1, PreS2) have been shown to modulate the antitumor immune response and suppress the growth of HCC in mice via increased IFN-γ production and an increase in the HBV-specific T cell population [168]. In addition, Glossogyne tenuifolia, a traditional, antipyretic and hepatoprotective herb, has been shown to inhibit the release of IFN-γ and IL-6 in LPS-activated PBMC from HCC patients, thus producing anti-inflammatory effects [169].

**GENE EXPRESSION STUDIES: THE CARCINOGENIC ROLE OF CYTOKINES IN THE MICROENVIRONMENT**

As the immune system is designed to eradicate damaged cells and tissues, what circumstances cause inflammation to promote cancer development rather than protect against it? In progressive stages of disease, are prometastatic changes inherent to the tumor cell, or are they acquired through time and/or influenced by environmental status such as the conditions of the metastasis sites? It is possible that neoplastic microenvironments favor polarized, chronic inflammatory states, which are protumorigenic rather than acute antitumor-immune responses that block tumor cells from escaping immune surveillance mechanisms. Cancer cells are capable of altering their environment through a variety of mechanisms, including the production of growth factors and proteolytic enzymes, which allow for disruption of tissue homeostasis and the creation of promigratory and proinvasive surroundings. However, tumors may also be directly affected by the tumor stroma itself, whereby tumor cells respond to certain factors present in the target organ that are permissive to and promote tumor extravasation, aggregation, and metastasis [170].

**Cytokine imbalances in the microenvironment**

The accumulated evidence has clearly demonstrated that immune cells in tumor-surrounding regions can influence tumor progression [171]. Our examination of the tumor microenvironment in HCC metastasis has indicated that the hepatic microenvironment from patients with HBV-positive metastatic HCC has a profound change in their gene expression profiles [172]. We found that a dominant, Th2-like cytokine profile, including an increase in IL-4, IL-8, IL-10, and IL-5 and a decrease in Th1-like cytokines including IFN-γ is associated with the metastatic phenotype. A unique cytokine signature was capable of predicting patients with HCC metastasis in a cross-validated study. Moreover, the prognostic performance of this signature was independent of and superior to other available clinical parameters for determining HCC patient survival or recurrence. We therefore suggest that the inflammatory status of the surrounding tumor milieu, in addition to the metastatic potential of the tumor cells, may play a prominent role in promoting HCC tumor progression and metastasis.

The predominant humoral cytokine response in the liver milieu of HCC metastasis patients suggests that shifts to anti-inflammatory/immune-suppressive responses may play a significant role in promoting HCC metastasis (Fig. 1). Although many immune cell types can produce and be activated by cytokines, T cells are the predominant cell type involved in this process. However, other cell types may also contribute to this finding. Macrophages are versatile and plastic cells that respond to microenvironmental signals with distinct, functional polarization programs, which regulate the influx of other im-
mune cells, such as T lymphocytes, by producing a variety of cytokines and chemokines [173]. Increased evidence indicates that tumor-associated macrophages (TAM) can polarize toward a type II phenotype, which is oriented toward the promotion of tissue remodeling and repair, a process that may be compatible with metastatic progression [171]. In addition, a clear role of B cells in metastasis has been demonstrated [174]. Our results indicate that the Th1-to-Th2-like profile switch in livers bearing metastatic HCC is accompanied by an overexpression of leukocytes including Kupffer cells. These findings are reminiscent of the TAM phenotype described above, whereby macrophages can potentially be alternatively activated. Consistently, we observed that an overexpression of CSF1, an activator and regulator of macrophages, accompanied the Th1-to-Th2-like profile switch in livers bearing metastatic HCC and could induce this profile in PBMC from healthy blood donors. Thus, CSF1 may play a prometastatic role in HCC.

Our findings support many published studies about tumor and stroma interaction [170], which suggest that the metastatic propensity of HCC is inherent to the tumor cell and influenced by the local environmental status of metastatic sites. The results described in this study may provide a new strategy for classification of patients and potential therapy of metastatic HCC by converting the unique Th2 to a Th1 profile. We speculate that post-surgical treatment with Th1-related cytokines in the Th2 group may ameliorate the metastatic-related imbalance of cytokines toward that of nonmetastatic HCC patients. Thus, a confident determination of individual HCC patients who have a Th1 or a Th2 profile may allow us to classify these patients in advance to determine those eligible and most likely to benefit from proinflammatory cytokine treatment. This possibility remains to be determined and could significantly affect the clinical outcome of patients likely to develop HCC metastasis.

PERSPECTIVE

Despite a multitude of studies, HCC remains a dismal disease for many individuals worldwide. Many of these studies have provided evidence that hepatitis viruses play roles in liver tumorigenesis by provoking immune-mediated liver injury and contributing to the inflammatory process. The balance between the extent of the infection and the quality and vigor of the antiviral response in patients may determine the severity and duration of viral hepatitis-induced liver injury.

Whereas the historical viewpoint was that host immunity is protective with regard to cancer, it is now clear that certain subsets of chronically activated immune cells promote growth and/or facilitate survival of neoplastic cells. Cytokines, in particular, have been demonstrated to play a significant role in HCC, as evidenced by the changes in their expression levels, affects on cellular pathways, and genetic imbalances. The particular role of any given cytokine can be specific or over-
lapping, thus generating downstream effects that result from a maze of complex interactions. Such an unexpected role for the immune response as enhancers of tumor physiology raises questions about how these tumor-promoting effects are conveyed and whether they can be harnessed to prevent or block these properties while simultaneously activating antitumor-immune responses.

The prevalence of metastasis heavily contributes to HCC mortality. A clinical challenge is to be able to identify cancer patients with metastatic potential in advance so that an appropriate, therapeutic regime can be applied. The published studies and current studies in our laboratory suggest that Th1 cytokines are involved in tumor development, whereas Th2 cytokines play a role in tumor progression. The identification of a cytokine-related molecular signature in primary tumors, which can predict metastasis and survival, has provided an opportunity to classify these HCC cancer patients in advance. Understanding the mechanisms underlying this process would allow us to develop an effective approach to reduce HCC-related mortality. Moreover, through interrogation of HCC genetics, cytokine SNPs may be used to define patient subgroups that may benefit from particular treatments. These types of strategies could allow for greater benefit of HCC patients by providing patient-tailored therapy. Current and new strategies and technological advances will undoubtedly enable future insight regarding the specific roles that the immune system plays in HCC. In sum, cytokines have been demonstrated to play a significant role in HCC. Despite a plethora of studies relating to cytokines and HCC, many of which have yielded results that are therapeutically promising, the full extent of the cytokine network and how it contributes to HCC remains to be understood.

REFERENCES

1. Balkwill, F., Mantovani, A. (2001) Inflammation and cancer: back to Virchow? Cancer Lett. 157, 539–545.
2. Ohno, S., Inagawa, H., Dhar, D. K., Fujii, T., Ueda, S., Tachibana, M., Suzuki, N., Inoue, M., Soma, G., Nagasue, N. (2005) The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients. Anticancer Res. 25, 5015–5022.
3. Coca, S., Perez-Piqueras, J., Martinez, D., Colmenarejo, A., Saez, M. A., Vallejo, C., Martos, J. A., Moreno, M. (1997) The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. Cancer 79, 2320–2328.
4. Ribatti, D., Ennas, M. G., Vaccara, A., Ferrelli, F., Nico, B., Ortu, S., Sirinpi, P. (2003) Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. Eur. J. Clin. Invest. 33, 420–425.
5. Leek, R. D., Landers, R. J., Harris, A. L., Lewis, C. E. (1999) Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. Br. J. Cancer 79, 991–995.
6. Imada, A., Shijubo, N., Kojima, H., Abe, S. (2000) Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. Eur. Respir. J. 15, 1087–1093.
7. Lewis, C. E., Pollard, J. W. (2000) Distinct role of macrophages in different tumor microenvironments. Cancer Res. 60, 605–612.
8. Coussens, L. M., Werb, Z. (2002) Inflammation and cancer. Nature 420, 860–867.
9. Balkwill, F., Charles, K. A., Mantovani, A. (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7, 211–217.
10. Finch, C. E., Crimmins, E. M. (2004) Inflammatory exposure and historical changes in human life-spans. Science 305, 1736–1739.
11. Endler, T., Gilleissen, S., Manis, J. P., Ferguson, D., Fleming, J., Alt, F. W., Mihm, M., Dranoff, G. (2003) Deficiencies of GM-CSF and interferon γ link inflammation and cancer. J. Exp. Med. 197, 1213–1219.
12. Dannenberg, A. J., Subharamaiah, K. (2003) Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. Cancer Cell 4, 431–436.
13. Hussain, S. P., Hofseth, L. J., Harris, C. G. (2003) Radical causes of cancer. Nat. Rev. Cancer 3, 276–285.
14. Wiltout, R. H. (2000) Regulation and antinastatic functions of liver-associated natural killer cells. Immunol. Rev. 174, 63–76.
15. De Lalla, C., Galli, G., Aldighetti, L., Romeo, R., Mariani, M., Monno, A., Nuti, S., Colombo, M., Calllea, F., Porcelli, S. A., Panina-Bordignon, P., Abignani, S., Casorati, G., Delabata, P. (2004) Production of proinflammatory cytokines by invariant NKT cells characterizes cirrhosis progression in chronic viral hepatitis. J. Immunol. 173, 1417–1425.
16. De Viessi, K. E., Coussens, L. M. (2005) The interplay between innate and adaptive immunity regulates cancer development. Cancer Immunol. Immunother. 54, 1143–1152.
17. Mann, J. R., Backlund, M. G., DuBois, R. N. (2005) Mechanisms of disease: inflammatory mediators and cancer prevention. Nat. Clin. Pract. Oncol. 2, 202–210.
18. Pisans, P., Parkin, D. M., Bray, F., Ferlay, J. (1999) Estimates of the worldwide mortality from 25 cancers in 1990. Int. J. Cancer 83, 18–29.
19. Tang, Z. Y. (2001) Hepatocellular carcinoma—cause, treatment and metastasis. World J. Gastroenterol. 7, 445–454.
20. Yuki, K., Hirohashi, S., Sakamoto, M., Kanai, T., Shimosato, Y. (1990) Growth and spread of hepatocellular carcinoma. A review of 240 consecutive autopsy cases. Cancer 66, 2174–2179.
21. Nakakura, E., K., Choti, M. A. (2000) Management of hepatocellular carcinoma. Oncology (Williston Park) 14, 1085–1098.
22. Amnola, M. (2004) Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J. Viral Hepat. 11, 333–393.
23. Tsai, J. F., Jeng, J. E., Ho, M. S., Chang, W. Y., Hsieh, M. Y., Lin, Z. Y., Tsai, J. H. (1997) Effect of hepatitis C and C virus infection on risk of hepatocellular carcinoma: a prospective study. Br. J. Cancer 76, 968–974.
24. Donato, F., Boffetta, P., Puslini, M. (1990) A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int. J. Cancer 45, 347–354.
25. Brochot, C., Jaffredo, F., Lagorce, D., Gerken, G., Meyer zum Buschfeldke, F., Papakonstantinou, A., Hadziyannis, S., Romeo, R., Colombo, M., Rodes, J., Buixi, J., Williams, R., Naoumou, N. (1998) Impact of HBV, HCV and GBV-C/HGV on hepatocellular carcinomas in Europe: results of a European concerted action. J. Hepatol. 29, 173–183.
26. Majos, M. E., Feinestone, S. M. (1997) The molecular viriology of hepatitis C. C. Hepatology 25, 1527–1539.
27. Di Bisceglie, A. M. (1998) Hepatitis C. Lancet 351, 351–355.
28. Stefollini, T., Andreone, P., Andriulli, A., Asicone, A., Craxi, A., Chiarantoni, M., Galante, D., Maghisi, O. G., Mazzanti, R., Medaglia, C., Pilleri, G., Rapaccini, G. L., Simonetti, R. G., Taliani, G., Tosti, M. E., Villa, E., Gasharrini, G. (1988) Characteristics of hepatocellular carcinoma in Italy. J. Hepatol. 9, 941–952.
29. Urawsina, T., Saigo, K., Kobayashi, S., Inasaki, H., Matsumura, H., Koidie, Y., Asano, T., Kondo, Y., Koike, K., Isono, K. (1997) Identification of hepatitis B virus integration in hepatocellular cancers infected hepatocellular carcinoma tissues. J. Hepatol. 26, 771–778.
30. Zhang, J. Y., Dai, M., Wang, X., Wu, L. Q., Li, D. S., Zhang, M. X., Wang, K. J., Dai, L. P., Han, S. G., Zhou, Y. F., Zhuang, H. (1998) A case-control study of hepatitis B and C virus infection as risk factors for hepatocellular carcinoma in Henan, China. Int. J. Epidemiol. 27, 574–582.
31. Sun, C. A., Farzaagedel, H., You, S. L., Lu, S. N., Wu, M. H., Wolfe, L., Hardy, W., Huang, G. T., Yang, P. M., Lee, H., Chen, C. J. (1996) Mutual confounding and interactional effects between hepatitis C and hepatitis B viral infections in hepatocellular carcinoma: a population-based case-control study in Taiwan. Cancer Epidemiol. Biomarkers Prev. 5, 173–178.
32. Tsai, J. F., Jeng, J. E., Ho, M. S., Chang, W. Y., Lin, Z. Y., Tsai, J. H. (1999) Hepatitis B and C virus infection as risk factors for hepatocellular carcinoma in Chinese: a case-control study. Int. J. Cancer 65, 619–621.
33. Donato, F., Tagger, A., Chiesa, R., Ribero, M. L., Tomasoni, V., Fasola, M., Gelatti, U., Portera, G., Boffetta, P., Nardi, G. (1997) Hepatitis B and C virus infection, alcohol drinking, and hepatocellular carcinoma: a case-control study in Italy. Brescia HCC Study. Hepatology 26, 579–584.
Wilson disease and hemochromatosis: oxyradical overload diseases. Proc. Natl. Acad. Sci. USA 97, 12770–12775.

Hagen, T. M., Huang, S., Curnutte, J., Fowler, P., Martinez, V., Wehr, C. M., Ames, B. N., Chisari, F. V. (1994) Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. Proc. Natl. Acad. Sci. USA 91, 1209–1210.

Kitasaka, S., Shinta, G., Kawasaki, H. (2003) Serum levels of interleukin-10, interleukin-12 and soluble interleukin-2 receptor in chronic liver disease type C. Hepatogastroenterology 50, 1569–1574.

Huang, Y. S., Hwang, S. J., Chan, C. Y., Wu, J. C., Chao, Y., Chang, F. Y., Lee, S. D. (1999) Serum levels of cytokines in hepatitis C-related liver disease: a longitudinal study. Zhonghua Yi Xue Za Zhi (Taipei) 62, 327–331.

Bortolami, M., Venturi, C., Giacomelli, L., Scalerta, R., Bacchetti, S., Marino, F., Floreani, A., Lise, M., Naccarato, R., Farinati, F. (2002) Cytokine, infiltrating macrophage and T cell-mediated response to development of primary and secondary human liver cancer. Dig. Liver Dis. 34, 794–801.

Nakazaki, H. (1992) Preoperative and postoperative cytokines in patients with cancer. Cancer 70, 709–713.

Sing, I. H., Binh, V. Q., Duy, D. N., Kun, J. F., Bock, T. C., Kremmers, P. G., Luty, A. J. (2003) Serum cytokine profiles associated with clinical presentation in Vietnamese infected with hepatitis B virus. J. Clin. Virol. 28, 93–103.

Sato, T., Asanuma, Y., Masaki, Y., Sato, Y., Hatakeyama, Y., Kusano, T., Kamada, K. (1994) Changes in tumor necrosis factor-α and interleukin-1β production following liver surgery on cirrhotic patients. Hepatogastroenterology 41, 1148–1153.

Wang, Y., Kato, N., Hoshida, Y., Yoshida, H., Taniguchi, H., Goto, T., Moriyama, M., Otsuka, M., Shina, S., Shiratori, Y., Ito, Y., Omatu, M. (2003) Interleukin-1β gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection. Hepatology 37, 63–71.

Tanaka, Y., Furuta, T., Suzuki, S., Orito, E., Yeo, A. E., Hiroshima, N., Sugachih, F., Ueda, R., Mizokami, M. (2003) Impact of interleukin-1β genetic polymorphisms on the development of hepatitis C virus-related hepatocellular carcinoma in Japan. J. Infect. Dis. 187, 1822–1825.

Hirankarn, N., Kimkong, I., Kummee, P., Tangkijvanich, P., Pooworaw, Y. (2006) Interleukin-1β gene polymorphism associated with hepatocellular carcinoma in hepatitis B virus infection. World J. Gastroenterol. 12, 776–779.

Ikeguchi, M., Hirooka, Y. (2005) Interleukin-2 gene expression is a new biological prognostic marker in hepatocellular carcinomas. Oncology 28, 255–259.

Morshed, S. A., Fukuma, H., Kimura, Y., Watanabe, S., Nishimaki, M. (1995) Interferon-γ, interleukin (IL)-2 and IL-2 receptor expressions in hepatitis C virus-infected liver. Gastroenterol. Jpn. 29, Suppl 5, 59–66.

Cao, Z. X., Chen, X. P., Wu, Z. D. (2003) Effects of splenectomy in patients with cirrhosis undergoing hepatic resection for hepatocellular carcinoma. World J. Gastroenterol. 9, 2460–2463.

Chia, C. S., Ban, K., Ilthim, H., Singh, H., Krishnan, R., Mokhtar, S., Malihan, N., Seow, H. F. (2002) Expression of interleukin-1β, interferon-γ and interleukin-10 in hepatocellular carcinoma. Immunol. Lett. 84, 163–172.

d’Arcville, C. N., Nouri-Aria, K. T., Johnson, P., Williams, R. (1993) Frequency and nature of cytokine gene polymorphisms in hepatocellular carcinoma in Hong Kong Chinese. Int. J. Gastroenterol. Cancer 34, 19–26.

Kroeger, K. M., Carville, K. S., Abraham, L. J. (1997) The −308 tumor necrosis factor-α promoter polymorphism effects transcription. Mol. Immunol. 34, 391–399.

Niro, G. A., Fontana, R., Goffreda, D., Valvano, M. R., Lacobelli, A., Facciorusso, D., Andriulli, A. (2005) Tumor necrosis factor gene polymorphisms and clearance or progression of hepatitis B virus infection. Liver Int. 25, 1173–1183.

Beckebaum, S., Zhang, X., Chen, X., Yu, Z., Frilling, A., Dvorak, G., Grosse-Wilde, H., Broelsch, C. E., Gerken, G., Cinacinati, V. R. (2004) Increased levels of interleukin-10 in serum from patients with hepatocellular carcinoma correlate with profound numerical deficiencies and immature phenotype of circulating dendritic cell subsets. Clin. Cancer Res. 10, 7200–7209.

Chen, C. Y., Wu, C. W., Liu, W. Y., Chang, T. J., Kao, H. L., Wu, L. H., King, K. L., Loong, C. C., Hsia, C. Y., Chi, C. W. (2000) Serum interleukin-10 but not interleukin-6 is related to clinical outcome in patients with resectable hepatocellular carcinoma. Ann. Surg. 231, 532–538.

Shin, H. D., Park, B. L., Kim, L. H., Jung, J. H., Kim, J. Y., Yoon, J. H., Kim, Y. J., Lee, H. S. (2003) Interleukin 10 haplotype associated with increased risk of hepatocellular carcinoma. Hum. Mol. Genet. 12, 901–906.

Hu, B. H., Lee, P. H., Yu, S. C. (1999) Secretion of acute-phase proteins before and after hepatocellular carcinoma resection. J. Formos. Med. Assoc. 98, 85–91.

Lee, Y., Park, U. S., Choi, I., Yoon, S. K., Park, Y. M., Lee, Y. I. (1998) Human interleukin 6 gene is activated by hepatitis B virus-X protein in human hepatoma cell lines. J. Viral Hepatol. 5, 479–484.

Kanaoka, Y., Yagi, T., Sadamori, H., Matsukawa, H., Matsuda, H., Inagaki, M., Ishikawa, T., Sato, I., Iwagaki, H., Tanaka, N. (2002) Analysis of host response to hepatocarcinoma by simultaneous measurement of cytokines in the portal vein, caval vein and radial artery. J. Int. Med. Res. 30, 490–305.

Ishikawa, M., Miyachi, T., Yagi, K., Chikashii, H., Fukuta, Y., Miyake, H., Harada, M., Yogoita, S., Tashiro, S. (1999) Clinical relevance of antibiotic-induced endotoxin release in patients undergoing hepatic resection. World J. Surg. 23, 75–79.

Reichner, J. S., Mulligan, J. A., Spinski, R., Sotomayor, E. A., Alhina, J. E., Bland, K. I. (1998) Effect of IL-6 overexpression on the metastatic potential of rat hepatocellular carcinoma cells. Ann. Surg. Oncol. 5, 279–286.

Cancell, U., Bukan, N., Sancak, B., Gunel, N., Ozinirel, S., Unal, A., Yucel, A. (2004) Serum hepcidin gene expression in patients with resectable liver tumors can distinguish patients with primary or metastatic liver tumors from those with benign liver lesions. Neoplasma 51, 209–213.

Park, B. L., Lee, H. S., Kim, Y. J., Kim, J. Y., Jung, J. H., Kim, I. H., Shin, H. D. (2005) Association between interleukin 6 promoter variants and chronic hepatitis B progression. Exp. Mol. Med. 37, 76–82.

Ryu, S. W., Kim, H. K., Rossmanith, W., Rutkay-Nebeday, B., Mallauer, L., Kammerer, B., Bursch, W., Schulte-Hermann, R. (1998) Levels of transforming growth factor β and transforming growth factor β receptors in rat liver during growth, regression by apoptosis and neoplasia. Hepatology 28, 717–726.

Jaskiewicz, K., Chasen, M. B. (1995) Differential expression of transforming growth factor α, adhesions molecules and integrins in primary, Budhu and Wang Cytokines and hepatocellular carcinoma 1211
metastatic liver tumors and in liver cirrhosis. Anticancer Res. 15, 559–562.

119. Yuen, M. F., Norris, S., Evans, L. W., Langley, P. G., Hughes, R. D. (2002) Transforming growth factor-β 1, activin and follistatin in patients with hepato-cellular carcinoma and patients with alcoholic cirrhosis. Scand. J. Gastroenterol. 37, 233–238.

120. Kim, Y. J., Lee, H. S., Int, J. M., Min, B. H., Kim, H. D., Jeong, J. B., Yoo, J. H., Kim, M. S., Kim, J. Y., Jung, J. H., Kim, L. H., Park, B. L., Shin, H. D. (2003) Association of transforming growth factor-β1 gene polymorphisms with a hepatocellular carcinoma risk in patients with chronic hepatitis B virus infection. Exp. Mol. Med. 35, 196–202.

121. Okumoto, K., Hattori, E., Tamura, K., Kiso, S., Watanabe, H., Saito, K., Sasaki, Y., Tsujiuchi, T., Murata, N., Tsutsumi, M., Konishi, Y. (2001) Alterations of the transforming growth factor-β signaling pathway in hepatocellular carcinomas induced endogenously and exogenously in rats. Jpn. J. Cancer Res. 92, 16–22.

122. Akhbar, S. M., Alee, M., Murakami, H., Tanimoto, K., Kumaqi, T., Yamashita, Y., Michtaka, H., Horike, N., Onji, M. (2001) Macrophage migration inhibitory factor in hepatocellular carcinoma and liver cirrhosis: relevance to pathogenesis. Cancer Lett. 171, 123–132.

123. Simonetti, R. G., Liberati, A., Angiolini, C., Pagliaro, L. (1997) Treatment of hepatocellular carcinoma: a systematic review of randomized controlled trials. Ann. Oncol. 8, 117–130.

124. Camma, C., Di Bon, D., Craxi, A. (2004) The impact of anti-trompic treatments on the course of chronic hepatitis C: an evidence-based approach. Curr. Pharm. Des. 10, 2123–2130.

125. Akiba, J., Yano, H., Ogasawara, S., Higaki, K., Kojiro, M. (2001) Expression and function of interleukin-8 in human hepatocellular carcinoma. Int. J. Oncol. 17, 257–264.

126. Shibaji, T., Kanamura, T., Ogawa, S., Nakano, H. (2000) The impact of interferon α-2b and α-2a treatment on chronic hepatitis C and liver tumors: a prospective randomized controlled trial study. J. Gastroenterol. 35, 387–394.

127. Asakawa, M., Kono, H., Amemiya, H., Matsuda, M., Suzuki, T., Maki, A., Fujii, H., Matsuura, Y., Kimura, S., Koike, K., Miyamura, T. (2002) Alteration of intrahepatic cytokine expression and AP-1 activation in transgenic mice expressing hepatitis C virus core protein. Virology 304, 41–52.

128. Perlermeter, G., Letteron, P., Carnot, F., Zavala, F., Pesaydre, D., Nalpas, R., Brecho, C. (2003) Alcohol and hepatitis C virus core protein additively increase lipolysis and synergistically trigger hepatic cytokine expression in a transgenic mouse model. J. Hepatol. 39, 1020–1027.

129. Sundstrom, S., Ota, S., Dimberg, I. Y., Masucci, M. G., Bergqvist, A. (2005) Hepatitis C virus core protein induces an anergic state characterized by decreased interferon-β2 production and perturbation of mitogen-activated protein kinase responses. J. Virol. 79, 2230–2239.

130. Majumder, M., Steele, R., Ghosh, A. K., Zhou, Y. X., Thornburg, L., Ray, R., Phillips, N. J., Ray, R. B. (2003) Expression of hepatitis C virus non-structural 3A protein in the liver of transgenic mice. FEBS Lett. 555, 526–532.

131. Yamada, T., Hanada, T., Tokuhisa, T., Kosai, K., Sato, M., Kohara, M., Yoshimura, A. (2002) Activation of STAT3 by the hepatitis C virus core protein leads to cellular transformation. J. Exp. Med. 196, 641–653.

132. Ohno, H., Kaneko, S., Lin, Y., Kobayashi, K., Murakami, S. (1999) Human hepatitis B virus X protein augments the DNA binding of nuclear factor-κB through its basic-leucine zipper domain. J. Med. Virol. 58, 11–18.

133. Yu, F. L., Liu, H. J., Lee, J. W., Liao, M. H., Shih, W. L. (2005) Hepatitis B virus X protein promotes cell migration by inducing matrix metalloproteinase-9. J. Hepatol. 42, 520–527.

134. Su, F., Theodoris, C. N., Schneider, R. J. (2001) Role of NF-κB and myc proteins in apoptosis induced by hepatitis B virus HBx protein. J. Virol. 75, 215–225.

135. Lin, S. J., Shu, P. Y., Chang, C., Ng, A. K., Hu, C. P. (2003) IL-4 suppresses the expression and the replication of hepatitis B virus in the hepatocarcinoma cell line HepB. J. Hepatol. 171, 4785–4796.

136. Chen, C. J., Yang, H. L., Su, J. C., Loo, C. L., You, S. L., Lu, S. N., Huang, G. T., Iloeje, U. H. (2006) Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 295, 65–73.

137. Montesquieu, J., Boura, P., Kouklakis, G. (2002) Locoregional immunomodulatory chemotherapy in hepatocellular carcinoma. Hepatogastroenterology 49, 1109–1112.

138. Wang, X. H., Qin, Y., Hu, M. H., Xie, Y. (2005) Dendritic cells pulsed with hsp70-peptide complexes derived from human hepatocellular carcinoma induce specific anti-tumor immune responses. World J. Gastroenterol. 11, 5614–5620.
157. Andrews, K. J., Ribas, A., Butterfield, L. H., Vollmer, C. M., Eilber, F. C., Dissette, V. B., Nelson, S. D., Shintaku, P., Mkhoubad, S., Nakayama, T., Taniguchi, M., GlaspY, J. A., McBride, W. H., Economou, J. S. (2000) Adenovirus-interleukin-12-mediated tumor regression in a murine hepatocellular carcinoma model is not dependent on CD1-restricted natural killer T cells. *Cancer Res.* 60, 6457–6464.

158. Margalit, M., Shibolet, O., Klein, A., Elinav, E., Alper, R., Thalenfeld, B., Engelhardt, D., Rabbani, E., Ilan, Y. (2005) Suppression of hepatocellular carcinoma by transplantation of ex-vivo immune-modulated NKT lymphocytes. *Int. J. Cancer* 115, 443–449.

159. Junbo, H., Li, Q., Zaide, W., Yunde, H. (1999) Receptor-mediated interleukin-2 gene transfer into human hepatoma cells. *Int. J. Mol. Med.* 3, 601–606.

160. Putzer, B. M., Stiewe, T., Rodicker, F., Schildgen, O., Ruhm, S., Dirsch, O., Fiedler, M., Damen, U., Tennant, B., Scherer, C., Graham, F. L., Roggendorf, M. (2001) Large nontransplanted hepatocellular carcinoma in woodchucks: treatment with adenovirus-mediated delivery of interleukin 12/B7.1 genes. *J. Natl. Cancer Inst.* 93, 472–479.

161. Peron, J. M., Couderc, B., Rochaix, P., Douin-Echinard, V., Asnacios, A., Souque, A., Voigt, J. J., Buscail, L., Vinel, J. P., Favre, G. (2004) Treatment of murine hepatocellular carcinoma using genetically modified cells to express interleukin-12. *J. Gastroenterol. Hepatol.* 19, 388–396.

162. Wang, Z., Qiu, S. J., Ye, S. L., Tang, Z. Y., Xiao, X. (2001) Combined IL-12 and GM-CSF gene therapy for murine hepatocellular carcinoma. *Cancer Gene Ther.* 8, 2173–2182.

163. Zager, J. S., Delman, K. A., Malhotra, K. A., Malhotra, S., Ehright, M. I., Bennett, J. J., Kates, T., Halterman, M., Federoff, H., Fong, Y. (2001) Combination vascular delivery of herpes simplex oncolytic viruses and ampicillin mediated cytokine gene transfer is effective therapy for experimental liver cancer. *Mol. Med.* 7, 561–566.

164. He, P., Tang, Z. Y., Liu, B. B., Ye, S. L., Liu, Y. K. (1999) The targeted expression of the human interleukin-2/interferon α2i fused gene in α-fetoprotein-expressing hepatocellular carcinoma cells. *J. Cancer Res. Clin. Oncol.* 125, 77–82.

165. Bui, L. A., Butterfield, L. H., Kim, J. Y., Ribas, A., Seu, P., Lau, R., GlaspY, J. A., McBride, W. H., Economou, J. S. (1997) In vivo therapy of hepatocellular carcinoma with a tumor-specific adenoviral vector expressing interleukin-2. *Hum. Gene Ther.* 8, 2173–2182.

166. Dumontier, J., Schonig, K., Oberwinkler, H., Low, R., Giese, T., Bujard, H., Schirmacher, P., Pfitzer, U. (2005) Liver-specific expression of interferon γ following adenoviral gene transfer controls hepatitis B virus replication in mice. *Gene Ther.* 12, 668–677.

167. Peng, B. G., Liu, S. Q., Kuang, M., He, Q., Totsuka, S., Huang, L., Huang, J., Lu, M. D., Liang, L. J., Leong, K. W., Ohno, T. (2002) Autologous fixed tumor vaccine: a formulation with cytokine-microparticles for protective immunity against recurrence of human hepatocellular carcinoma. *Jpn. J. Cancer Res.* 93, 363–368.

168. Gotsman, I., Alper, R., Klein, A., Rabbani, F., Engelhardt, D., Ilan, Y. (2002) Inducing oral immune regulation of hepatitis B virus envelope proteins suppresses the growth of hepatocellular carcinoma in mice. *Cancer* 94, 406–414.

169. Wu, M. J., Weng, C. Y., Ding, H. Y., Wu, P. J. (2005) Anti-inflammatory and antiviral effects of Glossogyne tenuifolia. *Life Sci.* 76, 1133–1146.

170. Mueller, M. M., Fusseneg, N. E. (2004) Friends or foes—bipolar effects of the tumor stroma in cancer. *Nat. Rev. Cancer* 4, 839–849.

171. Pollard, J. W. (2004) Tumor-educated macrophages promote tumor progression and metastasis. *Nat. Rev. Cancer* 4, 71–78.

172. Budhu, A., Forgues, M., Ye, Q. H., He, P., Zanetti, K. A., Kammula, U. S., Chen, Y., Tang, Z. Y., Wang, X. W. (2006) Prediction of venous metastases, recurrence and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment. *Cancer Cell* 10, 99–111.

173. Mantovani, A., Sozzani, S., Locati, M., Allavena, P., Sica, A. (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 23, 549–555.

174. De Visser, K. E., Korets, L. V., Coussens, L. M. (2005) De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* 7, 411–423.