REVIEW

Tumor microenvironment and hepatocellular carcinoma metastasis

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Abstract

The cross talk between tumor cells and the surrounding peritumoral stroma has been extensively studied as a dynamic system involving the processes of hepatocarcinogenesis, tumor invasion, and metastasis in recent few decades. Besides hepatocytes, liver tumor microenvironments are generally classified into cellular and noncellular components, including hepatic stellate cells, fibroblasts, immune, endothelial, mesenchymal stem cells, together with growth factors, cytokines, extracellular matrix, hormone as well as viruses et al. The noncellular components manipulate hepatocellular carcinoma invasion and metastasis by facilitating epithelial-mesenchymal transition, increasing proteolytic activity of matrix metalloproteinases, and regulating antitumor immunity, etc. Because the main cause of death in hepatocellular carcinoma patients is tumor progression with metastasis, a better understanding of the interplay between hepatocytes and their environment during tumor metastasis may be helpful for the discovery of novel molecular targets.

Key words

hepatocellular carcinoma, tumor metastasis, tumor microenvironent.

Accepted for publication 6 May 2013.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and the third most frequent cause of cancer death in the world, which accounts for 70–85% of primary liver cancer regarding the statistics data of American Cancer Society in 2007. The main cause of death in HCC patients is tumor progression with metastasis and invasiveness. Despite great efforts have been made to elucidate the molecular events underlying invasion and metastasis of HCC in the past several decades, the detailed mechanisms of tumor metastasis remain obscure.

The tumor microenvironment is a systematic concept that defines the behavior of cancer not by the genetics of the tumor cells alone but by the surrounding milieu. Immune cells and their mediators are present in the tumor microenvironment of most tumors. In addition, several literatures reported that other stromal components including fibroblasts, myofibroblasts, vascular endothelia cells, virus, as well as the surrounding extracellular matrix play synergetic roles on maintaining tumor environments. Recent studies unambiguously show that the cross talk between tumor cells and their surrounding microenvironments is necessary for cell survival, growth, proliferation, epithelial-mesenchymal transition (EMT), and metastasis.1,2

Because the majority of HCC patients have an underlying chronic inflammatory liver disease and inflammation is the main risk factor for the progression of HCC, it is necessary to clarify the underlying mechanisms for the maintenance and the dynamic manipulation of inflammable microenvironment during tumor progression and metastasis. In this review, we mainly summarize the current understanding of each component of the tumor microenvironment and their roles in the invasion and metastasis of HCC.
Distinct contributions of cells to HCC metastasis

**Hepatic stellate cells.** Hepatic stellate cells (HSCs) play an important role in hepatic injury and fibrosis, and are recently considered as an important component of the prometastatic microenvironment. In the tumor environment, HSCs undergo the transition from the “quiescent” to “activated” state and affect cancer cell proliferation and invasiveness in a paracrine manner. Although it has been suggested that HSCs might exert their prometastasis capability by producing a multitude of cytokines, chemokines, and growth factors, the molecular mechanisms underlying their affect on cancer cells are still poorly understood.

A unsupervised genome-wide expression profiling confirmed that the genes that signed the cross talk between HCC cells and HSCs were significantly enriched in the expression profiles of cirrhosis tissue from patients with metastasis, which may be targeted by epigenetic modulation. Another study has shown that besides several representative growth factors (including hepatocyte growth factor [HGF], stromal-derived factor-Ialpha (SDF-1), chemokine (C-C motif) ligand 5 (CCL5), osteopontin [OPN], and transforming growth factor-beta [TGF-B]) that have already been reported to be upregulated in tumor-associated stroma cells, epithormin (EPM) was also strongly elevated in cancer cell-activated HSCs as well as in HSCs of stroma surrounding HCC in clinical specimens. EPM is a morphogen expressed by HSCs and promotes HCC cell invasion and metastasis via the focal adhesion kinase–extracellular signal-regulated kinase–matrix metalloproteinase (MMP)-9 signaling pathway.

Moreover, the interactions between tumor HSCs (tHSCs) and immune cells also contribute to hepatic immune tolerance, and invasion and migration of HCC. For example, tHSC-induced T-cell hyposresponsiveness was associated with enhanced T-cell apoptosis and contributed to the migration and invasion of hepatoma cell. tHSCs were associated with markedly enhanced expression of B7-H1. Blockade of B7-H1/programmed death 1 (PD-1) ligation significantly reduced HSC immunomodulatory activity and hepatoma cell migration and invasion.

**Macrophages.** Macrophages exist in almost all tissues and play important roles in the maintenance of tissue homeostasis. The most frequently found immune cells within the tumor microenvironment are tumor-associated macrophages (TAMs) and T cells. TAMs mostly promote tumor growth and may be obligatory for angiogenesis, invasion, and metastasis. Recently, two groups have identified that HCC metastasis was associated with not only the inflammatory response but also with infiltration and the expression of macrophage colony-stimulating factor (M-CSF) in peritumoral liver tissue. High peritumoral M-CSF level and macrophage infiltration were associated with HCC progression, a high incidence of intrahepatic metastasis, and poor survival after HCC resection, highlighting the importance of TAM in the recurrence and metastasis of HCC. Pharmacological drugs, such as zoledronic acid combined with sorafenib, enhance antitumor effects in metastatic HCC by depleting the macrophage population.

Kupffer cells are specialized macrophages located in the liver lining the walls of the sinusoids that form part of the reticuloendothelial system (also called mononuclear phagocyte system). It has been reported that this kind of cells with expression of various cytokines played important roles for HCC progression. Lai et al. found that macrophage androgen receptor (AR) might influence wound healing via modulating tumor necrosis factor (TNF-α) expression, which could trigger p38 activation and played an important role in HCC metastasis. So, it can be assumed that AR in Kupffer cells may also contribute to the HCC progression.

Substantial clinical and experimental evidence suggests that TAMs can impel HCC progression through impairing cytotoxic PD-1+CD8+ T-cell immune responses, which is by expressing B7-H1 in HCC and mediating decreased effector function via interaction with PD-1 on effector T cells. OPN is a leading gene in HCC metastasis signatures and has been proved to play important roles in HCC metastasis. Kupffer cells can produce excessive OPN in response to inflammatory cytokine (interleukin [IL]-1, TNF-α, platelet-derived growth factor) stimulation, suggesting that a unique immunological condition regulated by Kupffer cells may promote HCC metastases.

**T cells.** T cells can exert both tumor-suppressive and promoting effects, as determined by their effector functions. A significant increase in the anti-inflammatory cytokines, such as IL-4, IL-5, IL-8, and IL-10 (Th2 cytokines), and a concomitant decrease in the pro-inflammatory cytokines such as TNF, interferon (IFN)-γ, and IL-1 (Th1 cytokines) were found in livers with metastatic HCC compared with the normal liver, suggesting that a stronger Th1-type immune response may, at least partially, inhibit tumor relapse.

The imbalance of immune cells in the tumor microenvironment is another important regulator of progression in HCC. Tumor-infiltrating lymphocytes, the primary immune component infiltrating solid tumors, are considered to be a manifestation of the host antitumor reaction. Regulatory T cells (Tregs), which account for 5–10% of all CD4+ cells, are presumed to act mostly in a protumorigenic fashion through the suppression of antitumor immune responses. Increased numbers of CD4+CD25+forkhead box P3 (FoxP3)+ Tregs infiltrating tumor cell nests have been demonstrated in HCC, which impair cytotoxic CD8+ T (cytotoxic T lymphocyte) cell proliferation, activation, degranulation, and production of granzyme A, granzyme B, and perforin.

Chen et al. found that FoxP3+ Tregs were highly aggregated and in an activated phenotype (CD69 [+] HLA-DR [high]) in the tumor site, where they can suppress the proliferation and IFN-γ secretion of CD4+CD25– T cells. The increased tumor-infiltrating Tregs predicted poorer prognosis in HCC patients.

Similar to those conclusions, Gao et al. have found that Tregs are associated with HCC invasiveness, and intratumoral balance of regulatory and cytotoxic T cells is a promising independent predictor for recurrence and survival in HCC. A combination of depletion of Tregs and concomitant stimulation of effector T cells may be an effective immunotherapy to reduce recurrence and prolong survival after surgery. The intratumoral CD45RO (+)/peritumoral CD57 (+) (memory/senescent T cell) ratio is of vital importance in preventing HCC extrahepatic metastasis and in particular demonstrates its independent prognostic value in liver transplant recipients. Further, accumulating results also suggest a critical role of the increased Tregs in dampening the natural killer (NK) cell immune response in HCC progression. Tregs selec-
tively express membrane-bound TGF-β, which downregulates NK group 2 member D expression on NK cells and suppresses NK cell effector functions, that is, homeostatic proliferation, cytotoxicity, and IL-12-mediated IFN-γ production. Recently, a complex differential regulation of human NK activity by tumor-induced Treg (iTreg) cells in the tumor microenvironment was reported. In contrast with the naturally occurring Treg cells, tumor iTreg cells inhibit IL-2-mediated NK cell activity in the absence of target cells, whereas enhance the tumoricidal activity of NK cells by target cell contact.

**NK and NKT cells.** The frequency and function of liver and peripheral NK cells have also been reported to be decreased in HCC patients. NK cells can inhibit liver tumor growth via production of IFN-γ and activation of NK cells. Impaired NK cell function and reduction of NK cells in cirrhotic liver are a major risk factor for developing HCC. However, there is also evidence suggesting that CD4 NKT cells may promote liver tumor growth via production of Th2 cytokine and subsequent inhibition of tumor antigen-specific CD8 T-cell expansion. Hence, further studies should be warranted to clarify the distinct roles of NKT cell subsets in HCC.

**Mesenchymal stem cells.** In response to inflammation, mesenchymal stem cells (MSCs) are known to migrate to tissue injury sites to participate in immune modulation, tissue remodeling, and wound healing. Tumors apply persistent mechanical and pathological stress to tissues and causes continual infiltration of MSCs. Until now, the role of MSCs in HCC metastasis remain obscure. Co-culture of MHCC97-H cells with MSC-conditioned medium revealed that MSCs enhance tumor growth but significantly inhibit the invasiveness and metastasis of HCC, possibly through downregulation of TGF-β1. On the contrary, MSCs pre-treated with pro-inflammatory cytokines (IFN-γ and TNF-α) lead EMT of HCC cells and upregulation of TGF-β in MSCs. The levels of MSCs expressing stage-specific embryonic antigen-4 (SSEA4) in clinical HCC samples significantly correlated with poor prognosis of HCC.

**Noncellular component involving HCC metastasis.**

Previous studies documented that many kinds of noncellular components of tumor microenvironment have participated in the tumor metastasis, especially HCC microvascular invasion, portal vein tumor thrombus, and distant metastasis. Apart from well-known metastatic-related noncellular components across various cancers, such as TGF-β, vascular endothelial growth factor, epidermal growth factor, and MMPs, other noncellular component factors have been extensively studied in HCC-related metastasis.

**HGF.** HGF is expressed in HSCs or myofibroblasts, and is thought to be a mediator of tumor-stromal interactions through which myofibroblasts increase the proliferation and invasion of HCC cells. Alternative splicing of the Kupffer-like factor 6 (KLF6) tumor suppressor into an antagonistic splice variant 1 (SV1) is a pathogenic event in several cancers including HCC. Elevated SV1 is associated with increased tumor metastasis and mortality. In HepG2 cells, HGF significantly enhances the ratio of SV1/KLF6 full by 40% through phosphorylation of Akt and subsequent downregulation of two splicing regulators, serine/arginine-rich splicing factor (SRSF) 3 (SRP20) and SRSF1. Moreover, the upregulation of HGF can promote HCC carcinogenesis and EMT via Akt and cyclooxygenase 2 pathways. Recent studies also reported that other molecules, including limited miR-198 and estrogen, could exert their capability of tumor metastatic suppressor by targeting HGF/MNNG HOS transforming gene (c-Met) pathway, suggesting HGF/c-Met may be a potential therapeutic target for personalized treatment in hepatocellular carcinoma.

**Hormones and their receptor.** The liver is a hormone-sensitive organ, and in fact, both normal liver and HCC tissues from male and female mammals have been shown to express specific estrogen receptors (ERs). Estrogens and androgenic steroids have both been related to development of liver tumors. But, the role of estrogen and androgens in HCC development and progression is still controversial. It seems that in the physiological status of premenopausal women, estrogen has a protective role against the development of HCC. On the contrary, the hyperestrogenic status of the cirrhotic male or the high concentrations of estrogens in the old formulations of oral contraceptives, together with the presence of variant ER in the liver and/or other risk factors, may increase the risk of developing HCC.

Previous reports showed that ERs are highly expressed in neoplastic liver, while increased ARs are associated with poor HCC prognosis. Recently, physiological doses of estrogen, no matter endogenous or exogenous, are demonstrated to suppress metastasis of HCC through modulation of inflammatory tumor microenvironment by suppression of HGF and IL-6 production. Estrogen attenuates HCC progression in vitro and in vivo, and this may contribute to the gender differences in HCC behavior. It modulates HCC malignancy by reducing tumor cell invasion, arresting cell cycle progression, and promoting apoptosis, characterized by decreased expression of MMP-2, MMP-9, proliferating cell nuclear antigen, cyclin A, cyclin D1, and B-cell lymphoma (Bcl)-2, and increased expression in cleaved caspase. Moreover, ER-α-mediated inhibition of nuclear factor (NF)-κB binding activity is also a pivotal event in this process.

Ma et al. demonstrated that hepatic AR may play dual roles in either promoting HCC initiation or suppressing HCC metastasis. Hepatic AR could enhance anoikis and suppress migration of HCC cells via suppression of p38 phosphorylation/activation and the NF-κB/MMP9 pathway, respectively. In addition, the preclinical trials also show that a combination therapy of increased AR expression and reduced multiple-kinase inhibitor (sorafenib) exhibited better therapeutic efficacy.

Additional, thyroid hormone receptors (TRs) play dual roles in manipulating HCC metastasis. TRs have the ability to induce TRAIL expression, and TNF-related apoptosis-inducing ligand (TRAIL), in turn, acts in concert with simultaneously synthesized Bcl-xL to promote metastasis, whereas the activation of TR/dickkopf 4 (DKK4)/Wnt/β-catenin cascade effectively inhibits the proliferation and migration of hepatoma cells. A somatostatin analog octreotide could prevent the occurrence of second primary hepatomas and lung metastasis after resection of primary HCC, suggesting that octreotide administration may be useful as an adjuvant therapy to improve survival of patients with HCC.
**Virus infection.** Among the well-known risk factors for HCC, chronic infection with hepatitis B virus (HBV) or hepatitis C virus is present in more than 85% of primary liver cancers.69 Despite the absence of a dominant oncogene encoded by HBV genome, HBV X protein (HBx), a key regulatory multifunctional protein of the virus, has been reported to exert a direct hepatocarcinogenic effect in the development of HCC.70 HBx deregulates a wide variety of host genes related to cell proliferation, cell cycle progression, apoptosis, metastasis, protein degradation pathways, and genetic stability.71 Previous studies have demonstrated that HBx is able to promote migration and invasion of hepatoma cells by upregulation of many proteins, such as forkhead box protein M1 (FoxM1), OPN, forkhead box protein M1 (FoxM1), MMPs, chemokine [C-X-C motif] ligand (MIG), and deregulation of intercellular adhesion.72–75

Further, a positive cross talk between HBx and the metastasis associated 1 (MTA1)/histone deacetylase (HDAC) complex in stabilizing hypoxia-inducible factor (HIF)-1α has been proven playing a critical role in angiogenesis and metastasis of HBV-associated HCC. HBx-induced deacetylation is important for proteasomal degradation of HIF-1α, and protein levels of MTA1 and HDAC1 are increased in the presence of HBx. The higher expression of HDAC1 in HCC was observed in human HBV-associated HCC specimens.76 In addition, HBx could exert its activity through enhancing the expression of miR-29a, which is involved in the regulation of migration of hepatoma cells.77

**Inflammatory cytokines.** Inflammatory microenvironment from chronic liver injury contributes to the development of hepatic fibrosis, cirrhosis, carcinogenesis, and eventually tumor metastasis. Not only parenchymal but also nonparenchymal cells can synthesize cytokines and be affected by cytokines in response to different stress stimuli. Besides TNF-α and IL-6,78 proven as the important mediators for HCC invasion, other cytokines have been reported involving the hepatocyte mesenchymal transition and metastasis recently. The higher frequency of IL-17A-positive cells was detected in tumor tissues in HCCs with metastasis, which could promote HCC metastasis by the upregulation of MMP2 and MMP9 expression by activating NF-κB signaling pathway.79 IL-18 not only directly inhibits HBV replication but also upregulates the messenger RNA levels of MMP-3 and MMP-9 in an NF-κB-dependent manner.80 Moreover, excessive IL-22 has been found in the HCC microenvironment, leading to tumor growth, inhibition of apoptosis, and promotion of metastasis because of signal transducer and activator of transcription 3 (STAT3) activation.81 The incidence of microscopic vessel invasion was significantly higher in IL-8-positive than in IL-8-negative tissues. More IL-8 was expressed in HCCs at pathological stages III/IV than in those at stages I/II. HepG2 cells showed more chemotactic and invasive activities in response to IL-8 stimulation.82

**Conclusion and future prospective**

In the past 10 years, we have learned a great deal about the importance of tumor microenvironments in HCC initiation, progression, invasion, and metastasis. The tumor microenvironment is a dynamic and active component rather than merely a fixed structure supporting tumor growth. Recently, several studies have found new molecules potentially involving the manipulation of tumor environment, including miRNAs microRNAs, LncRNA Long non-coding RNA (LncRNA), microvesicle, and metabolism. In addition, tumor-initiating cells also play the potential role in the tumor progression and invasion in HCC,83 suggesting that improving the knowledge on this relationship may be crucial for the identification of prognostic biomarker and designing the novel therapeutic methods in future.

**Acknowledgements**

This study was supported by grants: The State Key Project for Liver Cancer (2012ZX10002-009), the National Basic Research Program of China (2012CB316503), National Natural Science Foundation of China (30921006, 91029723), Science Foundation of Shanghai (10QA1408700 and 09CG33), and State Key Laboratory of Oncogenes and related Genes (91-10-02).

**Conflict of interest**

The authors have no potential conflict of interests to declare.

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Hepatocytes and their environment

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