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The Tumor Microenvironment in Hepatocellular Carcinoma: Current Status and Therapeutic Targets

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Abstract

A growing body of literature highlights the cross-talk between tumor cells and the surrounding peri-tumoral stroma as a key modulator of the processes of hepatocarcinogenesis, epithelial mesenchymal transition (EMT), tumor invasion and metastasis. The tumor microenvironment can be broadly classified into cellular and non-cellular components. The major cellular components include hepatic stellate cells, fibroblasts, immune, and endothelial cells. These cell types produce the non-cellular components of the tumor stroma, including extracellular matrix (ECM) proteins, proteolytic enzymes, growth factors and inflammatory cytokines. The non-cellular component of the tumor stroma modulates hepatocellular carcinoma (HCC) biology by effects on cancer signaling pathways in tumor cells and on tumor invasion and metastasis. Global gene expression profiling of HCC has revealed that the tumor microenvironment is an important component in the biologic and prognostic classification of HCC. There are substantial efforts underway to develop novel drugs targeting tumor–stromal interactions.

In this review, we discuss the current knowledge about the role of the tumor microenvironment in pathogenesis of HCC, the role of the tumor microenvironment in the classification of HCC and efforts to develop treatments targeting the tumor microenvironment.

Keywords

Hepatocellular carcinoma; tumor microenvironment; hepatic stellate cells; cancer-associated fibroblast; Kupffer cell; T cell; TGF- β 1; matrix metalloproteinase; tissue inhibitor of metalloproteinase; inflammatory cytokine; gene signature; treatment

1. Introduction

Hepatocellular carcinoma (HCC) is the seventh most common malignancy and the third leading cause of cancer-related death worldwide [1]. Despite the recent advances in diagnosis and treatment of HCC, it remains a highly lethal disease. The main cause of death

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in HCC patients is tumor progression with metastasis. However, the underlying mechanisms of tumor initiation, progression and metastasis are still not fully understood [2].

The majority of HCC patients have an underlying chronic liver disease; and liver cirrhosis is the main risk factor for the development of HCC[3,4]. Chronic liver injury is associated with dysregulated growth of hepatocytes and results in the formation of regenerative nodules, dysplastic nodules, and HCC. Nitta et al. demonstrated that cirrhotic liver-derived hepatocytes (CLDH) have a cellular signaling phenotype that indicates a change from a MAPK-independent cell survival pathway to a MAPK-dependent cell survival pathway. The CLDHs have increased vimentin and type 1 collagen expression, which are markers of mesenchymal cells, and morphologic features consistent with the epithelial-mesenchymal transition (EMT), a biologic process in which epithelial cells lose their phenotypic characteristics and acquire features typical of mesenchymal cells[5-7]. EMT is essential during embryonic development, tissue repair in the adult organism and cancer progression, and it is thought to be critical as a connection point between inflammation and the progression of degenerative fibrotic diseases and cancer[8].

Recent literature has highlighted the cross-talk between tumor cells and their surrounding microenvironments as well as a fundamental role of the tumor microenvironment in the pathogenesis of HCC. The tumor microenvironment plays a critical role in modulating the process of liver fibrosis, hepatocarcinogenesis, EMT, tumor invasion and metastasis. The tumor microenvironment largely consists of 1) cells such as hepatic stellate cells, fibroblasts, immune cells - including regulatory and cytotoxic T cells and tumor-associated macrophages, and endothelial cells, 2) growth factors including transforming growth factor β 1 (TGF- β 1) and platelet derived growth factor (PDGF), 3) proteolytic enzymes such as matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs), 4) extracellular matrix (ECM) proteins, 5) and inflammatory cytokines. In this review, we discuss the current understanding of each component of the tumor microenvironment and their roles in the pathogenesis of HCC. In addition, we examine current treatments targeting the tumor microenvironment as well as directions for future research.

2. Cells in the tumor microenvironment

2-1 Hepatic Stellate Cells (HSCs)

HSCs, which were once known as lipocytes, Ito cells, or peri-sinusoidal cells, are the major cell type responsible for collagen synthesis in the liver[9]. Hepatic stellate cells (HSC) are activated in response to liver damage and trans-differentiate into myofibroblast-like cells when liver injury is repeated, leading to the development of hepatic fibrosis [10,11]. HSCs undergo phenotypic transformation from quiescent, non-proliferating cells to proliferating, extracellular matrix (ECM) producing cells during the process of liver injury, which involves two steps. The initial phase is represented by the up-regulation of cytoskeletal protein expression including α -SMA, and the perpetuation phase is represented by the release of a multitude of cytokines, chemokines and growth factors [12-14]. Activated HSCs produce the extensive accumulation of ECM during liver fibrosis [11,15]. Activated HSCs also infiltrate the stroma of liver tumors and localize around tumor sinusoids, fibrous septa and capsules [16].

In addition to their role in development of liver fibrosis, activated HSCs promote HCC cell proliferation. Amann.T et al. demonstrated that the conditioned media collected from HSCs induce proliferation and migration of HCC cells cultured in monolayers and, moreover, they showed that in a 3-dimensional spheroid co-culture system, HSCs promote HCC growth and diminish the extent of central necrosis through the activation of NF kappa B and extracellular-regulated kinase (ERK) pathways [4]. Consistent with these findings,

simultaneous *in vivo* implantation of HSCs and HCC cells into nude mice promoted tumor growth and invasiveness, and inhibited necrosis. PDGF, TGF- β 1, MMP-9, JNK, insulin-like growth factor binding protein 5, cathepsins B and D, hepatitis B virus X protein, and HCV nonstructural proteins are all potent inducers of stellate cell activation, proliferation and collagen production, and therefore enhance liver fibrosis and hepatocarcinogenesis [5,11,17-23]. In contrast, adiponectin suppresses hepatic stellate cell activation and angiogenesis [24] (Figure 1).

2-2 Cancer-Associated Fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) are the most prominent cell type within the tumor stroma of many cancers (most notably breast and pancreatic carcinoma) and play a critical role in tumor-stromal interactions [25-27]. They are activated by TGF- β and are responsible for the synthesis, deposition and remodeling of excessive ECM, such as various types of collagen. CAFs modulate the biological activities of HCC. Mazzocca et al. showed that HCC cell growth, intravasation and metastatic spread are dependent upon the presence of CAFs and HCC cells reciprocally stimulate proliferation of CAFs, suggesting a key role for CAFs in tumor-stromal interaction [28]. CAFs from different tumor types express several growth factors, including hepatocyte growth factor (HGF), members of the epidermal growth factor (EGF), fibroblast growth factor (FGF) and Wnt families, and cytokines, such as stromal-derived factor (SDF)-1 α and IL-6 [27,29].

2-3 Lymphocytes and Kupffer Cells

The immune response in the tumor and tumor microenvironment is an important regulator of progression in many cancers, including HCC. Fu et al. showed that CD4(+)CD25(+) regulatory T cells were more predominant than CD8+ T cells in HCC tissues compared with adjacent benign tissue. They also demonstrated that CD4(+)CD25(+) regulatory T cells impair cytotoxic CD8+ T cell proliferation, activation, degranulation, and production of granzyme A, granzyme B, and perforin. In line with these findings, several studies found that low intratumoral CD8+ T cell and high regulatory T cell numbers are associated with a worse prognosis in HCC patients [30,31]. In addition, dysfunctional regulation of the immune response in the tumor microenvironment by excessive regulatory T cell activity, insufficient B7 costimulation, inhibition by specific ligands such as programmed death ligand-1, or TGF- β mediated impairment of CD8+ T cell anticancer functions are well known mechanisms by which cancers evade the immune response [32]. Similarly, increased densities of NK cells are associated with HCC cell apoptosis and decreased tumor cell proliferation[33].

Although Kupffer cells were initially thought to be involved in antitumor immunity, there is substantial clinical and experimental evidence that suggests that these tumor-associated macrophages (TAMs) enhance tumor progression by impairing cytotoxic CD8+ T cell immune responses[34]. Programmed death 1 (PD 1) is highly expressed in exhausted CD8+ T cells. Its interaction with programmed death ligand-1 (PD-L1) was shown to impair cytotoxic CD8+ T cell function in human HCC. Increased expression of PD-L1 in Kupffer cells is thought to mediate a PD1 and PD-L1 interaction that prevents the cytotoxic activities of CD8+ T cell against tumors. In fact, blocking the interaction between PD-L1 on Kupffer cells and PD1 on CD8+ T cells restores cytotoxic CD8+ T cell function[35]. Kupffer cells also produce IL-6 that stimulates the initiation and development of HCC from hepatocellular damage and compensatory proliferation[36]. Kupffer cells, as well as stellate cells, when activated by inflammatory cytokines (IL-1, TNF alpha, PDGF), produce excessive osteopontin that plays a pivotal role in various cell signaling pathways that promote inflammation, tumor progression and metastasis[37]. In Kupffer cells, NF-kappa B, the

master regulator of inflammatory and immune responses, is an important pathway for the integration of signals from the tumor microenvironment that promote carcinogenesis.

2-4 Endothelial cells and HCC

Endothelial cells in HCC tissues and normal tissues have molecular and functional differences. Tumor-associated endothelial cells have rapid cell turn over, enhanced motility, migration, and high expression of CD105 and TGF- β 1. Notably, TGF- β 1 plays the role of chemo attractant for CD105 expressing endothelial cells and thus promotes tumor angiogenesis. [38] Recent studies of isolated CD105+ endothelial cells from HCC, showed they had features of increased angiogenesis activity with higher resistance to chemotherapeutic agents and inhibitors of angiogenesis [39]. The expression of PDGF receptor alpha in tumor endothelial cells was reported to be associated with a high risk for metastasis [40].

3. Non-cellular components of the tumor microenvironment

3-1. TGF- β 1 and Other Growth Factors

The complex roles of TGF- β 1 in HCC have been extensively investigated. TGF- β 1 is released in the ECM in a latent form and activated by MMP-2 or MMP-9, which are richly expressed in the tumor microenvironment [41]. When activated, TGF- β 1 binds to TGF receptor II, phosphorylates TGF- β RI, and activates down stream signaling through Smad-2 and Smad-3. TGF- β 1 is up-regulated in HCC tissues and peri-neoplastic stroma and plays key roles in liver fibrogenesis and hepatocarcinogenesis [42]. TGF- β expression is markedly increased in the cirrhotic liver and is a potent inducer of stellate cell proliferation and collagen production [5,18]. JNK activity is required for TGF- β 1 induced HSC activation and proliferation. Kluwe et al. showed that pan-JNK inhibitors prevent PDGF-, TGF- β - and angiotensin II-induced murine HSC activation and decreased PDGF and TGF- β signaling in human HSC [20]. Connective tissue growth factor (CCN2, also known as CTGF), is a mediator of TGF- β action and plays an important role in HSC-mediated fibrogenesis [43].

Apart from its role in liver fibrogenesis, TGF- β plays a dual role in HCC pathogenesis. It normally acts as a tumor suppressor in the premalignant state through the inhibition of cell proliferation and activation of apoptosis signals. Anti-proliferative effects are mediated by the mobilization of cyclin-dependent kinase inhibitors and suppression of c-Myc while the proapoptotic mechanisms of TGF- β 1 are mediated by down regulation of anti-apoptotic proteins [44]. The tumor suppressor effect of TGF- β not only involves the hepatocyte itself, but also acts through the suppression of tumor stroma mitogens and tumorigenic inflammation [45]. The role of TGF- β 1 may shift from tumor suppressor to oncogenic growth factors via several different mechanisms[45,46]. It has been shown that HBx and HCV can shift hepatocytic TGF- β signaling from the tumor-suppressive pSmad3C pathway to the oncogenic pSmad3L pathway through the activation of c-Jun N-terminal Kinase (JNK) [47,48] (Figure 2). A recent study suggested that promoter methylation of tristetrapolin (TTP), a negative posttranscriptional regulator of C-Myc, shifts TGF- β 1 signaling in HCC tumorigenesis[49]. TGF- β 1 was also shown to up-regulate Snail and down-regulate E-cadherin, which is central evidence for the epithelial-mesenchymal transition process [50-52]. Consistent with this molecular evidence, TGF- β 1 increases migration, vascular invasion (by modifying the structure of β 1 integrin and increasing α 3 integrin expression), angiogenesis (by the production of VEGF), tumor-stromal cross-talk, and metastasis (by increasing connective tissue growth factor [28,53-57]). TGF- β 1 also facilitates the EMT process through the activation of the PDGF signaling pathway [58].

TGF- β 1 regulates oncogenic miRNA expression to promote HCC progression. Exposure of hepatocytes to TGF- β 1 increases miR-181b expression, which promotes cell growth,

survival, migration and invasion of HCC cells [59]. Similarly, TGF- β induces miR-23a, 27a, and 24 (clustered in 1 transcript on chromosome 19), which promotes growth and survival of HCC cells [60].

Other heparin binding growth factors such as PDGF, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) play important roles in HCC pathogenesis [17,61-63]. PDGF plays an important role in the transformation of HSC into myofibroblasts, thus promoting fibrogenesis in the liver and increasing cell proliferation. Campbell et al. showed that over-expression of PDGFC in the liver of the transgenic mouse results in HSC activation, proliferation, tissue fibrosis and subsequent development of hepatocellular carcinoma through the activation of the ERK-1/-2 and PKB/Akt signaling pathways [17]. As HCC is a highly vascular tumor, angiogenesis is a critical step in HCC progression. VEGF is a major growth factor that stimulates angiogenesis in normal and tumor tissues. In the inflammatory condition, the NF- κ B signaling pathway is activated, which increases VEGF expression[64]. VEGF acts not only on the proliferation of endothelial cells in the vasculature but also on the proliferation of cancer cells expressing VEGF-A receptor through downstream Akt/mTOR signaling[61]. FGFs are growth factors that are involved in tissue regeneration, wound healing, and angiogenesis [65,66]. Aberrant expression of FGFs has been reported in HCC, and it has been found to promote HCC and endothelial cell proliferation through the activation of downstream Erk and AKT pathways[67]. [M1] HGF is a growth factor expressed in hepatic stellate cells or myofibroblasts and is thought to be a mediator of tumor-stromal interactions through which myofibroblasts increase the proliferation and invasion of HCC cells [63].

3-2. Proteolysis enzymes

MMPs are zinc-dependent endopeptidases that were first described in the 1960s. MMPs play roles in physiologic tissue remodeling, development, and regulation during the inflammatory process[68,69]. There are a total of 23 known human MMPs, and different types of stromal and cancer cells produce various sets of MMPs. The main subtypes of MMPs are; 1) collagenases, MMP-1,-8,-13; 2) gelatinases, MMP-2,-9; 3) matrilysins, MMP-7,-26; 4) membrane type MMPs, MMP-14,-15,-16,-24,-17,-25; and 5) stromelysins, MMP- 3,-10,-11 [70].

MMPs play an important role in the development of liver cirrhosis. Mice with MMP-9 mutations have inhibited fibrogenesis, resulting in decreased portal and periportal accumulation of collagen. MMP-9 mutations suppress trans-differentiation of hepatic stellate cells to the myofibroblast-like phenotype *in vitro* and *in vivo*. Moreover, adenoviral application of the mutants MMP-9-H401A and -E402Q led to increased apoptosis of activated hepatic stellate cells, a main modulator of hepatic fibrosis [19].

MMPs lead to tissue remodeling, inflammation, tumor cell growth, migration, invasion and metastasis in many cancers, and they are also major modulators of the tumor microenvironment, playing key roles in HCC tumorigenesis[71]. Tumor invasion is coordinated by increased proteolytic activity of MMPs that degrade the surrounding stroma and allow tumor cell spread. Recent literature has shown that the role of MMPs is not only to degrade ECM but also to modulate cancer signaling pathways. It is well known that MMP-2, -9, and -14 activate TGF- β 1, which is a key modulator of epithelial-mesenchymal transition in HCC[72]. TGF- β 1 also reciprocally activates MMPs. miR-181b, which is up-regulated by TGF- β 1, up-regulates MMP-2 and -9 and promotes migration and invasion of HCC cells[59]. High expression of MMP-9 is associated with activation of the PI3K/PTEN/AKT/Mtor pathways in human HCCs[73,74]. MMPs also inhibit apoptosis signaling in cancer cells. For example, Fas ligand, which initiates the apoptosis process by binding Fas receptors, cleaved by MMP7 and is then unable to apoptosis[75]. MMP-2, -9, and -14

regulate the bioavailability of VEGF and promote angiogenesis in HCC cells [76,77]. MMPs are also involved in the modulation of the inflammatory response by regulating inflammatory cytokines and chemokines, which promote cancer progression [69,78,79]. MMP9 is highly expressed in HCC and its high expression is associated with capsular infiltration[80]. MMP-9 promotes HCC invasion and metastasis by cleaving the osteopontin precursor into an active form[81].

MMPs are released in inactivated forms due to the interaction between cysteine residue of the pro-domain and the zinc ion of the catalytic site[71]. Twist 1, focal adhesion kinase (FAK), claudin-1, HBV X protein, plasmin, furin, or other MMPs activate MMPs, thus promoting liver fibrosis and HCC progression, invasion and metastasis. [82-87] [88]. The chemopreventive effect of statins against HCC appear to be mediated by deactivation of MMP-2 and -9 due to decreased expression of MMP-14 and TIMP-2[89]. Phase III clinical trials are now ongoing to compare the efficacy of sorafenib alone and sorafenib coupled with pravastatin (NCT01075555).

Active MMPs are regulated by a negative feedback loop to prevent excessive tissue damage and inflammation. MMP activity is regulated at the level of gene transcription, by activation and deactivation of proteolytic enzymes, and by natural inhibitors called TIMPs. TIMPs play complex roles in regulating cell proliferation, apoptosis, MMP activation, and angiogenesis as well as in preventing the excessive degradation of ECM. TIMP3 is a negative regulator of MMPs and is known to inhibit tumor progression, invasion, and metastasis in HCC[90,91]. High expression of TIMP1 suppresses the proliferative and invasive potential of HCC cell lines [92,93]. Also of note is ability of TIMP2 to activate as well as inhibit MMPs. At high concentrations, TIMP2 inhibits MMP2 activation while at lower concentrations, it activates MMP2 by triggering MMP2 and MT1-MMP clustering, which is the critical step in MMP2 activation[94,95]. The enzymatic activities of MMP and TIMP are tightly balanced, and high MMP activity, especially involving MMP-2 and -9, is associated with tumor invasion, metastasis and a poor outcome in HCC [74].

3-3. Extracellular Matrix Proteins

The ECM consists of fibrous proteins and proteoglycans, which have a protein core to which glycosaminoglycans (GAGs) are attached during their synthesis. The main roles of proteoglycans are to maintain the structural framework of the tissue and to store growth factors within the ECM. Heparan sulfate, chondroitin sulfate, and keratan sulfate are the major types of proteoglycans in the ECM. Of these, heparan sulphate proteoglycans (HSPGs) are known to play an important role in the pathogenesis of HCC as key growth factors such as FGF, HGF, PDGF, and VEGF are either stored in HSPGs or utilize HSPGs as co-receptors for binding to their tyrosine kinase receptors [17,62,63]. The sulfation of particular saccharide moieties of HSPGs is required for growth factor signaling. Our previous studies have shown that the heparin-degrading endosulfatases, sulfatase 1 (SULF1) and sulfatase 2 (SULF2), play important roles in modulating these heparin-binding growth signaling pathways[96-99]. Although SULF1 and SULF2 are structurally very similar, FGF signaling and its downstream AKT/mitogen-activated protein kinase pathway is activated by SULF2 but abrogated by SULF1[98,100]. Desulfation of co-receptor type HSPGs by SULF1 inhibits binding of the growth factor to its receptor, abrogating growth factor signaling and producing a tumor suppressing effect [101]. On the other hand, desulfation of HSPGs by SULF2 releases growth factors from the storage subtype of HSPGs and increases binding of growth factors to their receptors, leading to the activation of growth signaling[96]. PI-88, a heparan sulfate mimetic synthesized for targeting heparanases in cancer, has been shown to inhibit SULFs activity [102]. The safety and efficacy of PI-88 as an adjuvant therapy for hepatocellular carcinoma after curative resection was shown recently in a phase II clinical trial [103].

Laminins are cell adhesion proteins in the ECM that form a web-like structure to resist tensile forces in the basal lamina. They consist of three α , β and γ chains, and 15 different heterodimers have been characterized[104]. Of the different subtypes of laminins, laminin-5 is expressed in HCC nodules, and its expression is associated with the metastatic phenotype of HCC[105]. Laminin-5 (Ln-5), together with TGF- β 1, was reported to promote EMT [51]. Integrin α 3 β 1-and α 6 β 4-mediated adhesion, proliferation, migration and invasion of HCC cells are dependent upon Ln-5 [106-109].

Integrins are surface receptor proteins that mediate cell-matrix and cell-cell adhesion. There are more than 20 integrin heterodimers due to alternative splicing and combinations of α and β subunits[110]. β 3 integrin was shown to be associated with inhibition of cell growth and promotion of apoptosis[111], and over-expression of β 1 integrin inhibits HCC cell proliferation by preventing Skp-2 dependent degradation of p27 via PI3K pathways[112]. Enhanced expression of α 3 β 1 integrin is associated with increased migration and invasion of HCC cells[109]. Collagens are the most abundant protein in the ECM and provide a structural support for cells. They also promote cell migration and proliferation in HCC. Let-7g, a known tumor suppressor miRNA, down-regulates COL1A2 and inhibits HCC cell migration and growth[113].

3-4 Inflammatory Cytokines

Inflammatory milieu from chronic liver injury contributes to the development of hepatic fibrosis and eventually, HCC. IL-6, TNF- α , and IL-1 are well-established mediators of HCC progression in liver inflammation. IL-6 is a multifunctional inflammatory cytokine produced by Kupffer cells in the liver in response to hepatocyte death that contributes to compensatory hepatocyte proliferation[114]. Serum IL-6 is increased in cirrhosis and high serum IL-6 is associated with increased risk for HCC and a poor prognosis in patients with HCC[115-118]. Estrogen suppresses IL-6 production in Kupffer cells, partly explaining the gender discrepancy in HCC development [119]. A recent study also showed that IL-6 is a link between obesity and HCC as increased expression of IL-6 and TNF in obese mice leads to the activation of the IL-6 signaling pathway via the downstream STAT3 and ERK pathways, thus promoting tumorigenesis in the liver[120].

TNF- α is a multifunctional cytokine produced mainly by Kupffer cells and other immune cells and is an essential cytokine for liver regeneration following liver injury due to the activation of its downstream NF-KB and Akt pathways[121]. Similarly, IL-1 is a pro-inflammatory cytokine that promotes MyD88 adaptor protein-dependent compensatory proliferation of hepatocytes[82]. IL-1 also promotes HSC proliferation, activation, and trans-differentiation into the myofibroblastic phenotype in addition to activating HSCs to produce and activate MMPs, particularly MMP9 [122].

IL-12 is an immune response mediator which induces the production of interferon gamma from NK cells or naïve T cells, promotes helper T cell differentiation, enhances cell-mediated immune responses, and activates cytotoxic lymphocytes[123]. The antitumor effect of IL-12 is thought to be mediated by the activation of tumor specific cytotoxic T lymphocytes and NK cells, and inhibition of angiogenesis. Intra-tumoral injection of IL-12 gene therapy induced lymphocyte infiltration into the tumor and inhibited tumor growth and angiogenesis in a mouse model[34,124]. The use of IL-12 in clinical practice is limited due to the severe systemic toxicity resulting from high interferon gamma levels in large doses[125] and the minimal efficacy of low doses[126].

4. Tumor microenvironment: Prognostic gene signatures

Since the early 2000s, global gene expression profiling of HCC has provided new insights into the molecular and prognostic classification of HCC. Various subtypes of HCC were defined that have distinctive tumor biologies and altered cell signaling pathways as well as different prognoses. Most importantly, these studies have consistently revealed the significance of the tumor microenvironment in the biological and prognostic classification of HCC.

For TGF- β , consistent with the dual role of TGF- β in HCC pathogenesis, global gene expression profiling of human HCC showed that TGF- β gene signatures can cluster into two homogeneous groups of HCC with early or late TGF- β signatures. The late TGF- β signature is associated with an invasive HCC phenotype and increased risk of tumor recurrence[127]. A recent meta-analysis of gene expression profiling from eight independent HCC cohorts proposed three subclasses of HCC, one of which was characterized by TGF- β -induced Wnt activation and the enrichment of gene sets associated with the EMT process[128]. MMPs and TIMPs have been included in gene signatures linked to poor prognosis. MMP14 was one of the signature genes associated with HCC vascular invasion in humans[129]. Lee et al. integrated gene expression data from rat fetal hepatoblasts and adult hepatocytes with HCC from human and mouse models. HCCs were classified into mature hepatocyte and immature hepatoblast subtypes. MMP1 and TIMP1 were signature genes in the immature hepatoblast subtypes of HCC that was associated with a poor prognosis[130].

The importance of inflammatory cytokine profiles in the tumor microenvironment has also been recognized in gene expression profiling. Functional enrichment analysis with Gene Ontology categories showed the enrichment of chemotaxis and humoral immune response genes as well as proliferation and development-related functions in the group at high risk of recurrence after surgical resection of HCC [83]. Gene expression signatures from the adjacent benign tissue were reported to predict late recurrence of HCC, this signature was characterized by inflammation-associated pathways and growth factors including NF- κ B, TNF- α , and IL-6[131]. IL-6, a major inflammatory cytokine was one of the signature genes in the hepatoblast phenotype signature [130]. In line with this result is the finding that inflammation and immune response-related gene signatures with an increase in Th2 cytokines in adjacent benign tissue can predict venous metastases, recurrence, and prognosis in patients with HCC [132]. Osteopontin, secreted from Kupffer or stellate cells in response to inflammatory cytokines, was also reported to be a leading gene in HCC metastasis signatures[133].

5. Tumor-Stroma interaction: A New Therapeutic Target for HCC

As most systemic chemotherapies fail to improve overall survival in patients with advanced HCC, efforts to develop new drug treatments have shifted from systemic chemotherapy to targeted treatment against the tumor-stromal interaction. The basic rationale for targeting tumor-stromal interaction is to suppress the effect of surrounding tissues or cell types that stimulate hepatocarcinogenesis, tumor progression, invasion, and metastasis while minimizing systemic toxicity by delivering drug effects specifically to tumors and their microenvironment. Each component of the tumor microenvironment shares some functional redundancies. Therefore, targeting one molecular component of the tumor microenvironment does not necessarily suppress HCC progression. For example, with MMPs, several enzymes display proteolytic activities toward the same ECM proteins[134,135]. Therefore, current drugs mostly target the tumor-stromal interaction by inhibiting receptors and their downstream signaling pathways, thereby abrogating the

cancer-promoting signaling provided by the tumor stroma rather than directly targeting specific components of the tumor stroma.

Sorafenib, an oral multi-kinase inhibitor, is the most successful medication of this kind. It inhibits VEGFR-2/-3 and PDGFR as well as Raf kinase, disrupting tumor-stromal interactions and resulting in decreased cell proliferation and angiogenesis. The efficacy and safety of sorafenib have been demonstrated in Phase III clinical trials, and it is currently the standard of care for patients with advanced stage HCC[131,136]. Similarly, brivanib, which targets VEGFR2 and FGFR, sunitinib, which targets PDGFR, VEGFR, C-KIT and FLT-3, erlotinib, which targets EGFR, linifanib, which targets VEGFR and PDGFR, ramucirumab, which targets VEGFR2, and PI-88, which targets heparanase as well as sulfatases, are now in Phase III clinical trials for the treatment of HCC (Table 1).

Targeted treatment against TGF- β signaling appears to be promising as high expression of TGF- β is a key mediator of liver fibrosis, HCC progression, and the EMT process in addition to being a poor prognostic indicator of HCC. TGF- β receptor 1 kinase inhibitor (LY2109761) deactivates Smad-2, decreasing the migration and (vascular) invasion of HCC cells and up-regulating E cadherin expression in HCC cell membranes, which mediates cell adhesion[28,53]. More recently, LY2109761 was shown to inhibit tumor specific neoangiogenesis by blocking paracrine cross-talk between HCC and endothelial cells via Smad 2 dependent inhibition of VEGF production with an efficacy that was surprisingly superior to bevacizumab, which specifically targets VEGF [56]. In addition, LY2109761 was also shown to interrupt the cross-talk between HCC cells and cancer-associated fibroblasts through the down-regulation of connective tissue growth factor (CTGF), thus inhibiting tumor progression[57]. Phase I clinical trials targeting TGF- β signaling for the treatment of HCC have not yet been performed.

6. Future prospective and Conclusion

There have been substantial advances in the understanding of the importance of the tumor microenvironment in HCC initiation, progression, invasion, and metastasis over the past few decades. The tumor microenvironment changes dynamically and consequently affects HCC behavior. It is now being recognized as an active component of the tumor rather than merely a passive structural support of tumor growth. In this regard, treatments against the tumor microenvironment and its interaction with HCC cells are under active investigation. Although targeting one specific element of the tumor microenvironment is often ineffective due to the functional redundancies of each component of the tumor microenvironment, targeted treatments (i.e. sorafenib) against tumor-stromal interaction through the inhibition of growth factor receptors have become the standard treatment for advanced stage HCCs in clinical practice. A better understanding of the biological and molecular interactions between each element of the tumor microenvironment and the tumor cells is critical in elucidating the heterogeneous biologic features of HCC and identifying additional effective treatment targets. This insight has the potential to eventually translate into improvements in clinical practice ranging from the prevention and prognostication of HCC to prolonging the survival of patients with advanced stage HCC.

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Abbreviations

ECM	extracellular matrix
FGF	fibroblast growth factor
HCC	hepatocellular carcinoma
HGF	hepatocyte growth factor
HSPG	heparan sulfate proteoglycan
HS	heparan sulfate
IL	interleukin
MMP	matrix metalloproteinase
MyD88	myeloid differentiation factor 88
NF-κB	nuclear factor kappa B
PDGF	platelet- derived growth factor
STAT3	signal transducer and activator of transcription 3
Sulf1	sulfatase 1
Sulf2	sulfatase 2
TGF	Transforming growth factor
TNF-α	Tumor necrosis factor- α
VEGF	Vascular endothelial growth factor

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Figure 1. Regulators of stellate cell activation and their roles in liver fibrosis and carcinogenesis Stellate cells and CAF activated by several factors induce proliferation, invasion and metastasis in hepatocellular carcinoma. TGF- β plays a critical role in liver fibrosis and tumorigenesis through the epithelial-mesenchymal transition.

Figure 2. Modulation of TGF- β signaling by Hepatitis B or C

TGF- β normally suppresses cell proliferation and induces cell apoptosis through the activation of the pSmad3C mediated p21^{WAF1} pathway and down regulation of anti-apoptotic proteins. HBx proteins and HCV impair pSmad3C/p21^{WAF1} tumor suppressive pathways and activate JNK/pSmad3L oncogenic pathways, which in turn activate c-myc and related oncogenic pathways. This also leads to increased angiogenesis via VEGF, vascular invasion through the activation of β 1 integrin, and promotion of EMT.

Table 1

Clinical trials targeting the tumor-stromal interaction for the treatment of HCC

Treatment	Phase	Target	Trial ID
Brivanib	3	VEGFR2, FGFR1	NCT00858871
Linifanib	3	VEGFR, PDGFR	NCT01009593
Sorafenib	3	VEGFR, PDGFR, Raf	NCT00492752
Sunitinib	3	VEGFR, PDGFR, c-KIT	NCT00699374
Ramucirumab	3	VEGFR2	NCT01140347
Erlotinib	3	EGFR	NCT00901901
PI-88	3	Heparanase, SULFs	NCT00568308
Bevacizumab	2	VEGF	NCT00162669
Cediranib	2	VEGFR, PDGFR, c-KIT	NCT00238394
BIBF-1120	2	VEGFR, PDGFR, FGFR	NCT01004003
E-7080	2	VEGFR, FGFR, PDGFR, c-KIT	NCT00946153
TSU-68	2	VEGFR2, FGFR, PDGFR	NCT00784290
XL-184	2	VEGFR2, MET, RET	NCT00940225
Vandetanib	2	VEGFR, EGFR	NCT00508001
Cetuximab	2	EGFR	NCT00142428
BIIB-022	2	IGF-1R	NCT00956436
Cixutumumab	2	IGF-1R	NCT00639509
CT-011	2	PD-1/2	NCT00966251
MEDI-575	1	PDGFR	NCT01102400
BAY73-4506	1	VEGFR, PDGFR, FGFR-1, Raf, RET, c-KIT	NCT01117623
GC33	1	GPC3	NCT00976170
AVE1642	1	IGF-1R	NCT00791544
Liver NK cell	1	Liver NK cell inoculation	NCT01147380