World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v20.i15.4128

World J Gastroenterol 2014 April 21; 20(15): 4128-4140
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (1): Hepatocellular carcinoma

Role of the tissue microenvironment as a therapeutic target in hepatocellular carcinoma

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Supported by EU-Marie Curie Initial Training Network (ITN), FP7-PEOPLE-2012-ITN 2012, Grant Agreement No. 316549
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Received: October 6, 2013 Revised: January 11, 2014
Accepted: February 16, 2014
Published online: April 21, 2014

Key words: Tissue microenvironment; Hepatocellular carcinoma; Transforming growth factor-beta; Laminin-5; Cancer stem cells; Therapy; Target therapy

Core tip: We discuss a new hypothesis for therapeutic approaches in hepatocellular carcinoma (HCC). This novel idea is to regard the entire liver as responsible for the onset, growth and progression of HCC. In this scenario, we focus on the tissue microenvironment components as an ideal target for systemic therapies, taking into account the tumor/host interactions.

Rani B, Cao Y, Malfettone A, Tomuleasa C, Fabregat I, Giannelli G. Role of the tissue microenvironment as a therapeutic target in hepatocellular carcinoma. *World J Gastroenterol* 2014; 20(15): 4128-4140 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i15/4128.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i15.4128

Abstract

Hepatocellular carcinoma is difficult to treat, primarily because the underlying molecular mechanisms driving clinical outcome are still poorly understood. Growing evidence suggests that the tissue microenvironment has a role in the biological behavior of the tumor. The main clinical issue is to identify the best target for therapeutic approaches. Here, we discuss the hypothesis that the entire tissue microenvironment might be considered as a biological target. However, the tissue microenvironment consists of several cellular and biochemical components, each of which displays a distinct biological activity. We discuss the major components of this environment and consider how they may interact to promote tumor/host crosstalk.

INTRODUCTION

The tissue microenvironment consists of a dynamic population of cellular and non-cellular components which form a multifaceted regulatory network that helps to maintain the homeostasis of an organ. The liver microenvironment consists of a heterogeneous multitude of several components including extracellular matrix (ECM) components (laminin, fibronectin, collagen and proteoglycans), immune cells, Kupffer cells, endothelial cells, cytokines, fibroblasts and various growth factors[1]. These normal cellular and non-cellular microenvironment components are not only essential for the normal physiological and biological behavior of an organ, but are critical in opposing resistance to malignant cell growth[2] (Figure 1A).

Fundamentally, a tumor and its microenvironment have a mutual influence on each other's fate. Accumulations of mutations in normal cells bring them to a benign tumor state, where they stay dormant because they lack the main hallmark of cancer, namely the ability to invade, metastasize and form vasculature (angiogenesis)[3,4]. These mutual interactions between the mutated cells and the microenvironment modulate the ECM composition; they activate fibroblasts, recruit immune or inflammatory cells and pericytes, and stimulate endothelial cells to invoke angiogenesis, stirring the cascade of various cytokines, chemokines and growth factors[5,6]. This interactive, complex communication between the tumor and its microenvironment components favors cancer progression.

During hepatocellular carcinoma (HCC) progression, as the microenvironment components continue to interact with each other as well as with HCC cells, they acquire an abnormal phenotype due to tissue remodeling, which contributes to modulate the biological behavior of the tumor and thus facilitates cancer progression and metastasis.

The abrupt annual increase in HCC incidence in recent years of more than 750000 cases worldwide has constantly driven efforts to find new potential therapies for HCC[7,8]. Although surgical resection and liver transplantation are the so-called "curative treatments", the limiting factors are a shortage of healthy donor livers as compared to the emerging cases of HCC, and the fact that in advanced stages of HCC, surgery is not possible. Therefore, many studies have been focused on tumor-destructive approaches. The neovascularized nature of HCC is a potential target for the use of systemic therapies that can impede aberrant molecular pathways. The use of small molecule multi-kinase inhibitors such as Sorafenib has resulted in a significant improvement in overall survival of patients with advanced HCC[9,10]. Sorafenib targets the vascular endothelial growth factor receptor (VEGFR), and various other multi-kinase inhibitors targeting VEGFR are undergoing clinical trial for the treatment of HCC, such as sunitinib, axitinib, linifanib, pazopanib, vandetanib, cediranib and regorafenib, as well as monoclonal antibodies such as bevacizumab[11]. Another approach, namely transarterial chemoembolization (TACE), induces tumor hypoxia and this upregulates angiogenic factors such as VEGF[12,13]. TACE does not induce complete necrosis and after treatment the peripheral area of the tumor becomes viable again due to re-vascularization[14]. Combination therapy has been tried, but several studies failed to observe any survival benefits after the use of TACE with anti-angiogenic agents such as Sorafenib[15]. Cetuximab, a monoclonal antibody against the epidermal growth factor (EGFR), failed to show any significant activity against HCC in a phase Ⅱ study[16]. Clinical trials using small molecules targeting EGFR, such as erlotinib, gefitinib and lapatinib, were also ineffective against HCC[17-19]. A phase Ⅱ clinical trial of a multi-targeted agent, namely Dovitinib, which targets VEGFR, platelet-derived growth factor receptor and fibroblast growth factor receptor, is now under way[20]. Apart from genetic defects, epigenetics also play a pivotal role in hepatocarcinogenesis. *In vitro* and *in vivo* data have shown that the use of a histone deacetylase (HDAC) inhibitor (HDACi), along with dihydroartemisinin (DHA), elicited antitumor activity in liver cancer[21]. Recently, a phase I dose escalation trial of an HDACi, CHR2845, against cancer-associated inflammation in HCC was started in the United Kingdom. A pre-clinical Phase I / Ⅱ study of the HDACi, PXD101 (belinostat), has also been conducted and showed a good safety profile in HCC patients[22].

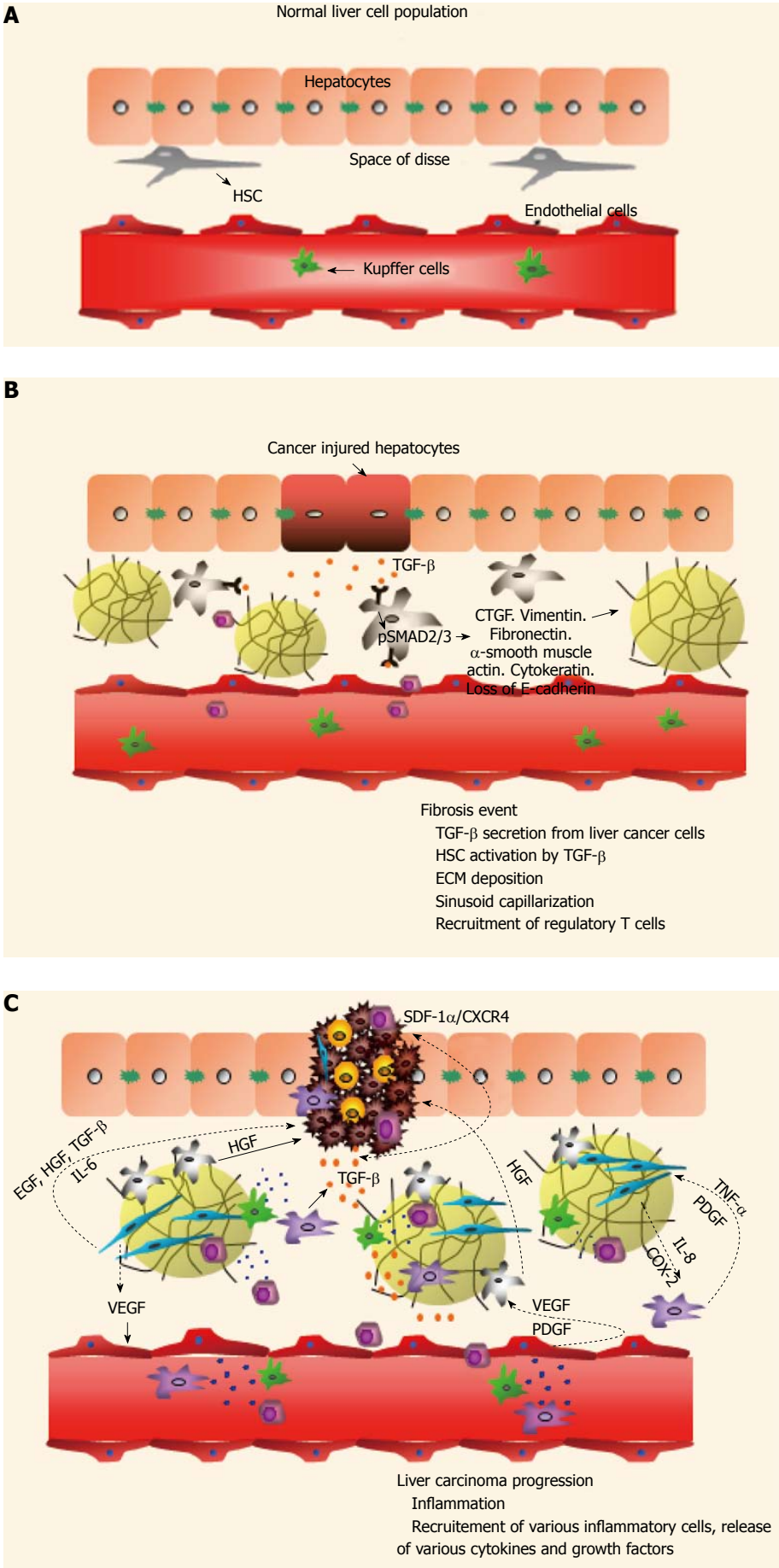
Due to the lack of early stage HCC diagnostic markers and efficient chemo-preventive strategies to limit HCC progression once cirrhosis is established, the survival rate of HCC patients is still poor and they mainly die of tumor progression and metastasis. Tumor heterogeneity is also a crucial barrier to HCC treatment, as tumor cells become resistant to chemotherapies[23]. In this scenario, it seems interesting to analyze the results of a potential therapeutic approach to HCC consisting of targeting the tumor tissue microenvironment. Tissue microenvironment components are genetically stable and are less likely to evolve into a drug-resistant phenotype, therefore it would be easier to target these components than tumor cells, which are genetically unstable and chemotherapy-resistant[24]. Additionally, tumor stroma exerts tumor-suppressing as well as tumor-promoting signals. A pancreatic cancer mouse model showed that inhibition of the Hedgehog signaling pathway reduced the level of tumor-associated stroma and improved the vascular delivery of gemcitabine[25]. Nevertheless, although several theories have been proposed to explain the role of stroma in carcinogen-induced tumors, the actual relationships are not yet proven. In addition, stromal cells may be a target for carcinogens, inducing either new cancers, or metastatic growth[26].

A better understanding of the complex network of interactions between tumor cells and their milieu could offer new insight into novel targets for HCC treatment. In this review, we have updated the literature and discussed the various issues with the aim of shedding further light on the role of the tissue microenvironment as a therapeutic target in HCC.

CELLULAR COMPONENTS OF THE TISSUE MICROENVIRONMENT

Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) are the major component of the tumor microenvironment and play a crucial role in tumor-stromal interactions[27-29]. CAFs promote tumor progression, invasion and chemoresistance to clinical therapies[28,30,31]. The origin of CAFs in HCC is controversial and various studies have revealed multiple origins, including the trans-differentiation of hepatic stellate cells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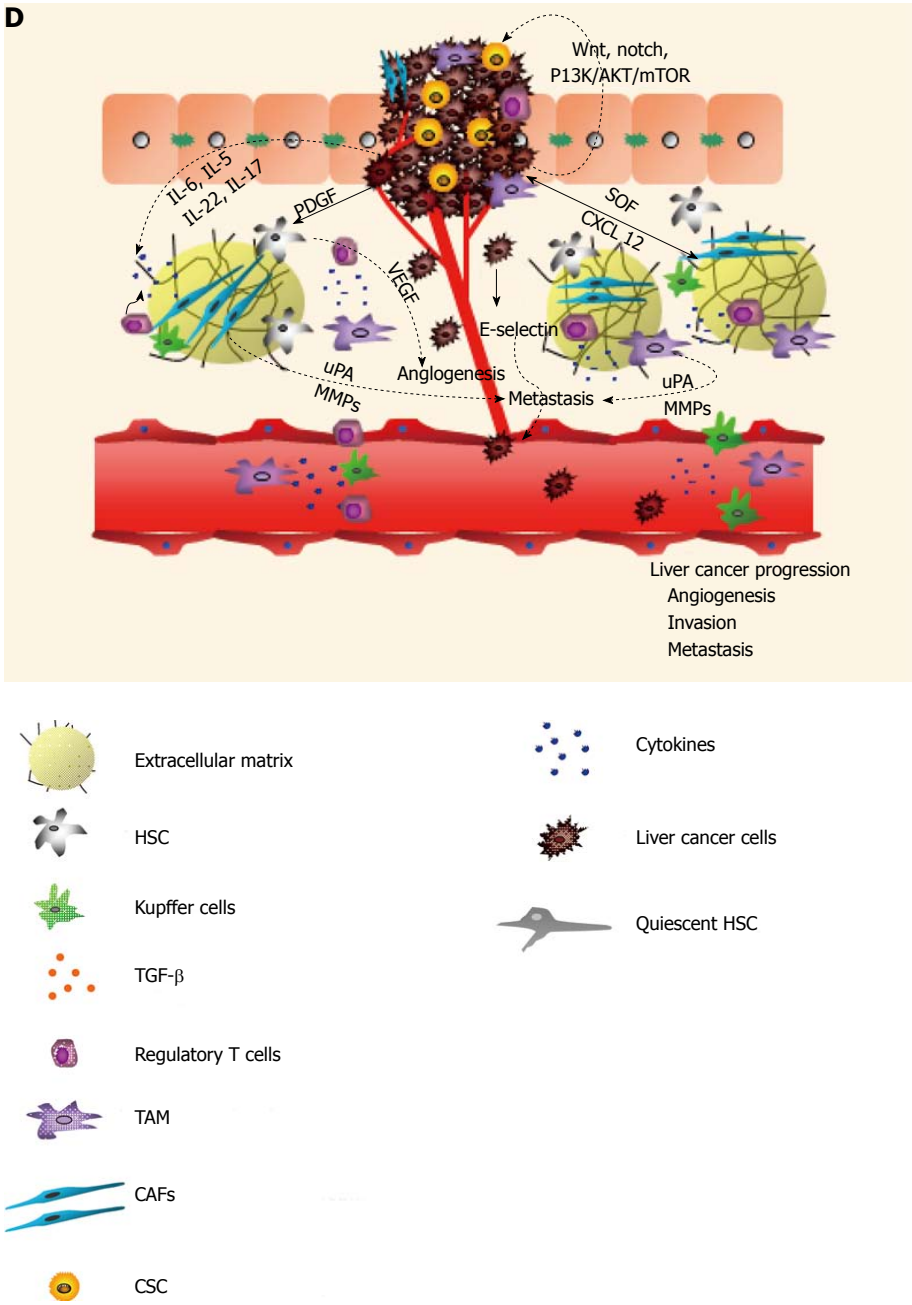


Figure 1 Progression of hepatocellular carcinoma: Crosstalk between hepatocellular carcinoma and its milieu. A: Healthy liver cell population. The normal liver cell population consists of quiescent hepatic stellate cell (HSC), Kupffer cells, and fenestrated endothelial cells which allow the exchange of blood and substrates between the space of Disse and hepatocytes; B: Fibrotic liver and its microenvironment. Fibrotic liver features cancer-injured hepatocytes, which trigger the release of transforming growth factor-β (TGF-β), defenestration of endothelial cells and recruitment of regulatory T-cells. Binding of TGF-β to its receptor on HSC triggers phosphorylation of SMAD2/3 signaling, which activates HSC to secrete extracellular matrix (ECM) components such as vimentin, CTGF, cytokeratin and muscle actin; C: Progression of liver cancer and its interaction with the milieu. Malignant hepatocytes proliferate in an uncontrolled manner. Infiltration of immune cells causes inflammation. Malignant hepatocytes secrete TGF-β which binds to, and activates, HSC. Activated HSC deposit more ECM. Recruitment of immune cells and cancer-associated cells elicits a signaling cascade. Compressed air foam system (CAFs) secrete vascular endothelial growth factor (VEGF) to stimulate endothelial cells to induce angiogenesis. In turn, endothelial cells secrete VEGF and platelet-derived growth factor (PDGF), which triggers the release of hepatocyte growth factor (HGF) from HSC. HGF secreted by HSC promotes malignant hepatocyte proliferation. Also, PDGF induces the differentiation of HSC into myofibroblasts, which cause fibrosis and the development of HCC. Activated CAFs also secrete EGF, HGF, TGF-β and interleukin-6 (IL-6) to aid cancer cell proliferation. CAFs produce cyclo-oxygenase-2 (COX-2) and IL-6 to induce tumor-associated macrophages (TAMs) production. Activated TAMs release TNF-α and PDGF to reinforce CAFs activation. Stromal cell-derived factor-1α (SDF-1/CXCL12) and its receptor CXCR4 are crucial in cancer stem cell (CSC) interactions with their surroundings. TGF-β upregulates CXCR4 expression in liver cancer cells and allows them to migrate to SDF-1α enriched niches. D: Progression and growth of liver carcinoma. Angiogenesis, Invasion and Metastasis are the crucial hallmarks of cancer. In HCC, HSC secrete VEGF to promote angiogenesis. CAFs and TAMs secrete various uPAs and matrix metalloproteinases (MMPs) to induce metastasis. Cancer cells secrete E- selectin to induce metastasis. Cancer stem cells activate the Wnt, Notch, phosphoinositide 3-kinase/Protein Kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway and thus contribute to the molecular heterogeneity of liver cancer. CAFs secrete SDF/CXCL12 to induce proliferation and invasion of liver tumor cells. Cytokines such as IL-6, IL-5, IL-22 and IL-17, produced by T regs or other immune cells, aid in liver cancer proliferation, angiogenesis and metastasis.

(HSC) during liver injury, activation of resting fibroblasts and a direct contribution of hepatocytes through the epithelial to mesenchymal transition (EMT)[32]. CAFs modulate the biological activity of HCC cells, as documented by the demonstration that lysophosphatidic acid induces HCC progression by recruiting peri-tumoral fibroblasts and promoting their trans-differentiation into myofibroblasts. In addition, activated CAFs create a favorable tumor environment by modulating immune cells such as NK cells. NK cells have anti-tumor activity, but this is significantly reduced in HCC[33]. Thus, activated CAFs remodel the ECM, which facilitates the release of various cytokines and growth factors and as a result magnifies HCC growth. Transforming growth factor-β (TGF-β) activates CAFs, which display α-smooth muscle actin (α-SMA), fibroblast activation protein, fibroblast surface protein, and vimentin[27,29] expression. Because CAFs also enhance the metastatic potential of HCC cells, targeting TGF-β receptor type I using LY2109761 can down-regulate CTGF production, which in turn interferes with the crosstalk between CAFS and HCC cells, and hence inhibits stromal growth and metastasis[34] (Figure 1B). These findings clearly demonstrate that CAFs have an important role in overall cancer progression and hence are the significant modifiers of cancer evolution. In certain types of cancers, CAFs regulate cancer stem cells (CSCs), and since CSCs are resistant to chemotherapies it seems possible that this is one reason why HCC is difficult to treat, also due to its high recurrence rate, CAFs may regulate the stemness of HCC. Further exploration of the interactions between CAFs and HCC cells may help to identify novel HCC targets.

HSC

HSC, also known as lipocytes or Ito cells, are multifunctional cells that perform several vital functions, including Vitamin A storage to maintain retinoid homeostasis[35], the production of matrix metalloproteinases (MMPs) and ECM components such as collagen to remodel the ECM[36], the production of various cytokines[37] (interleukin-6 (IL-6), interleukin-1β (IL-1β), chemotactic peptide-1, chemokines[38] (chemokine (C-C motif) ligands 5 and 21 (CCL5), (CCL21) and growth factors (such as TGF-α, TGF-β), epidermal growth factor (EGF), platelet derived growth factor and basic fibroblast growth factor[39]. HSCs display different phenotypes according to their morphology, functions and gene expression. The normal healthy liver stores the quiescent phenotypic state of HSCs, however, massive liver injury activates HSCs, leading to a cascade of various cytokines, ECM components and the up-regulation of cytoskeletal proteins such as α-SMA[40]. Activated HSCs can proliferate through the action of potent inducers such as cathepsins B and D, hepatitis virus B and C, PDGF, TGF-β1, MMP-9, JNK, insulin-like growth factor binding protein 5, non-structural proteins that induce liver fibrosis and hepatocarcinogenesis, while adiponectin, for instance, suppresses HSC activation[41].

Apart from their active contribution in liver cirrhosis, these activated HSCs or myofibroblasts also infiltrate the stroma of liver tumors, and when confined around the tumor sinusoids, fibrous septa and capsule, they assist in HCC progression[42] (Figure 1C). *In vivo* studies further support these data and showed that HSCs and HCC cells implanted into nude mice promote tumor growth and invasiveness by activating nuclear factor-κB and extracellular regulated kinase (ERK) in HCC cells[42]. HSCs produce and secrete laminin-5 (Ln-5), which induces HCC migration by activating the mitogen-activated protein kinase (MEK/MAPK)/extracellular-signal-regulated kinase (ERK) pathway, but not the phosphoinositide 3-kinase (PI3K/Akt) pathway[43].

Being multifunctional in nature, HSCs also act as liver-specific pericytes and promote tumor vascularity[44]. Pericytes are characterized by the expression of PGDF receptors; similarly, HSC cells produce PGDF and express PGDF receptors during liver injury[45,46]. Tumors and endothelial cells secrete PGDF to stimulate and recruit pericytes and so induce angiogenesis; pericytes secrete VEGF to support neovascularization[47]. These multifunctional phenotypes of HSC display a pivotal role, suggesting that they may be a valid therapeutic target in HCC treatment.

Tumor-associated macrophages

Macrophages are circulatory immature monocytes released from bone marrow which travel through the blood circulation to reach their destined tissue, where they mature and undergo differentiation into resident macrophages, such as Kupffer cells in the liver. Tumor-associated macrophages (TAMs) are the major inflammatory cells that infiltrate tumors. Tumor-derived signals such as macrophage-colony stimulating factor (M-CSF or CSF-1), VEGF, macrophage inflammatory protein 1a (MIP-1a), CCL3, CCL4, CCL5, CCL8, and angiopoietin-2, attract TAMs into the tumor microenvironment[48]. Different microenvironment signals determine distinct polarized activation states of macrophages, namely the classically activated (M1) and the alternatively activated (M2) phenotypes. Lipopolysaccharides (LPS) and Th-1 cytokine interferon-γ (IFN-γ) exposure polarizes macrophages to the M1 phenotype, whereas Th-2 cytokines interleukin-4 (IL-4), IL-10, and IL-13 exposure polarizes macrophages to the M2 phenotype[48,49]. The M1 phenotype displays high levels of antigen-presenting cells with an increased expression of IL-12, whereas the M2 phenotype shows low levels of antigen-presenting cells with a distinctive expression of various cytokines such as IL-10 and TGF-β[50]. In the tumor microenvironment, TAMs are mostly polarized towards the M2 phenotype, with a high expression of IL-10, arginase I, IL-6 and low expression of IL-12, tumor necrosis factor (TNF) and proinflammatory cytokines such as nitric oxide (NO) and reactive oxygen species (ROS)[50-52]. An increased number of TAMs is correlated with tumor cell proliferation, angiogenesis, metastasis and a poor prognosis[53]. Indeed, depletion

of macrophages by sorafenib significantly inhibits tumor progression, angiogenesis and metastasis[54]. Hence, TAMs are clearly essential for tumor growth. In human HCC, activated TAMs are localized within the peritumoral stroma and display strong expression of human leukocyte antigen (HLA-DR), IL-6, IL-1β and IL-23, whereas within the cancer niche, TAMs exhibit low expression of HLA-DR and IL-10[55]. Additionally, HCC cells recruit TAMs by secreting VEGF, TGF-β, PDGF, CCL2 and M-CSF and by expressing glypican-3[29,56,57]. TAMs secrete various cytokines, which contribute to the pathogenesis of HCC. For instance, in a diethylnitrosamine (DEN)-induced HCC mouse model system, DEN exposure promoted the release of IL-6 from Kupffer cells in response to IL-1β from damaged hepatocytes. Then, IL-6 promoted abnormal proliferation of the surviving hepatocytes by triggering the Signal Transducer and Activator of Transcription 3 (STAT-3) and Extracellular-signal-Regulated Kinases (ERK) pathways, which in turn control target genes involved in both cell proliferation and survival, thus contributing to HCC progression[58]. Moreover, TAMs-derived cytokines and growth factors induce immune suppression and contribute to tumor growth. IL-10 and TNF-α, secreted by TAMs in an autocrine manner, stimulate B7-H1 expression on macrophages and impair $CD8^+$ T-cell activity, which allows immune escape of tumor cells[59]. Since TAMs are the main infiltrating cells in the tumor microenvironment, therefore, they may be a potential target for clinical therapy. A macrophage activation state within peritumoral and tumoral tissue is a potent prognostic factor. TAMs secrete numerous cytokines, chemokines and growth factors, which support HCC progression as these TAMs-derived signals modulate the interaction with tumor cells. Moreover, TAMs support angiogenesis, metastasis, and cancer progression. Therefore, the complex network of tumor-stroma crosstalk could be a promising target of therapy for HCC.

Tumor-associated endothelial cells

The morphology of tumor vasculature is different from that of normal vessels. Tumor vessels are irregular, incomplete and fenestrated blood vessels with an irregular blood flow and increased permeability; this is possibly due to the molecular and functional differences between tumoral and normal endothelial cells[60,61]. Tumor-associated endothelial cells (TAECs) are cytogenetically abnormal[62] with a rapid turnover rate, enhanced mobility, migration and high expression of the endoglin marker (also known as CD 105 or TGF-β receptor)[57]. TAECs also contain several secretory organelles such as Weibel-Palade bodies, tissue plasminogen activator (tPA) organelles and type-2 chemokine containing organelles. These organelles secrete tPA, cytokines IL-8 and IL-6, monocyte chemoattractant protein-1 (MCP-1), and growth-regulated oncogene-α (GRO-α)[57,63]. Insufficient radiofrequency ablation (RFA) enhances TAEC migration and angiogenesis and promotes invasiveness of the residual HCC[64] (Figure 1D).

NON-CELLULAR COMPONENTS IN THE TISSUE MICROENVIRONMENT

TGF-β structure and receptor

TGF-β is a cytokine and belongs to the TGF-β cytokines superfamily, which also includes activins, inhibins, Mullerian inhibitor substance (Mis) and bone morpho-genetic proteins (BMPs). TGF-β is a multifunctional cytokine that can control proliferation, cellular differentiation, adhesion, migration, apoptosis and other functions in most cells[65,66]. In mammals, TGF-β exists in three isoforms, TGF-β1, TGF-β2 and TGF-β3. TGF-β1 is the most abundant, showing the highest expression[65]. TGF-β can inhibit the proliferation of most cell types, especially epithelial cells. Furthermore, it enhances the proliferation of mesenchymal cells, producing the ECM, as well as inducing fibrosis[67]. There are two main receptors for TGF-β, namely TGF-β receptor Ⅰ or activin receptor-like kinase (ALK), and TGF-β receptor Ⅱ. In mammals, 7 types of receptor Ⅰ and 5 types of receptor Ⅱ have been found[68].

ECM

ECM contains various proteoglycans, glycoproteins, polysaccharides and water[69]. Chemotaxis induces cell adhesion to the ECM, which is mediated by ECM receptors such as integrins, discoidin domain receptors and syndecans, as well as to various ECM components including fibronectin, laminin, collagens and elastin[70]. Ln-5 is a cell adhesion glycoprotein belonging to the Laminin family which forms a mesh-like structure to resist the tensile forces in the basal lamina. Each laminin is a heterodimer and consists of α3, β3 and γ2 chains encoded by three different genes, LAMA3, LAMB3 and LAMC2, respectively[71]. Ln-5 with the γ2 chain is a marker of invasiveness in several carcinomas, suggesting its role in tumor cell spread[72-74]. Ln-5 is not detected in normal liver, but shows high expression in HCC nodules, associated with a more proliferative and metastatic phenotype. The presence of the Ln-5 γ2 chain in metastatic HCC is correlated with poor prognosis and survival[75,76]. In HCC, invasive tumor cells secrete TGF-β1, which triggers invasiveness and motility in Ln-5 by inducing the expression of the transmembrane integrin receptor α3β1. Ln-5 also plays a crucial role in the epithelial to EMT, which is crucial in tumorigenesis. In HCC, Ln-5 upregulates the expression of the transcriptional repressors Snail and Slug, which induce the EMT[77,78]. Ln-5, together with TGF-β1, promotes the EMT in HCC by over-expressing Snail and Slug and downregulating E-cadherin, followed by translocation of β-catenin to the nucleus[78]. Gefitinib, a specific inhibitor of EGFR, suppresses HCC growth by inhibiting phosphorylation of the receptor and subsequently the Akt and Erk1/2 pathways in HCC cells *in vitro*. However, the presence of Ln-5 in HCC antagonises gefitinib's efficacy in a dose-dependent manner, suggesting a potential

drug (gefitinib) failure in Ln-5 positive HCC cases[79].

CANCER STEM CELL NICHE AS A NEW PLAYER IN THE HCC MICROENVIRONMENT

Cancer stem cells in liver tumors

Constant proliferation of stem cells occurs in renewing liver tissues, where mutations expand the altered stem cells, perpetuating and increasing the likelihood of additional mutations and tumor progression. Overall, many data indicate that liver stem/progenitor cells follow their own rules and regulations. The same signals that are essential for their activation, expansion and differentiation are good candidates for the role of contributing, under suitable conditions, to the paradigm of transformation from a pro-regenerative to a pro-tumorigenic role[80] (Figure 2A, B). The fact that about 40% of HCCs are clonal suggests that these tumors may originate from stem cell-like precursors. Recent evidence supports the existence of CSCs in HCC[81], which can be identified by several cell markers, including CD133, the epithelial cell-adhesion molecule EpCAM, as well as CD90, CD44, CD13, OV6, ALDH enzymatic activity, and the side population of cells (SP) revealed by Hoechst dye staining[82]. Indeed, the presence of EpCAM+ cells has been associated with a poorly differentiated morphology and high serum alpha-fetoprotein levels in HCC, whereas the presence of CD90+ cells was associated with a high incidence of distant organ metastasis[83]. Furthermore, circulating stem cell-like EpCAM+ tumor cells indicate a poor prognosis in HCC patients after curative resection. High CD44 standard isoform (CD44s) expression is associated with the EMT profile and with intrahepatic dissemination of HCC after local ablation therapy[84]. The expression of CD133 confers a malignant potential by regulating metalloproteinases in HCC cells. Importantly, CD13 is a marker for semiquiescent CSCs in human liver tumor cell lines and clinical samples. CD13+ cells predominate in the G0 phase of the cell cycle and typically form cellular clusters in cancer foci. Following treatment, these cells survived and were particularly abundant along the fibrous capsule where liver cancers usually relapse[85]. From the mechanistic standpoint, CD13 reduced reactive oxygen species (ROS)-induced DNA damage after genotoxic chemo/radiation stress and protected cells from apoptosis. In any case, these cells are likely resistant to targeted therapies such as sorafenib[86].

MECHANISM OF THE TGF-α SIGNALING PATHWAY

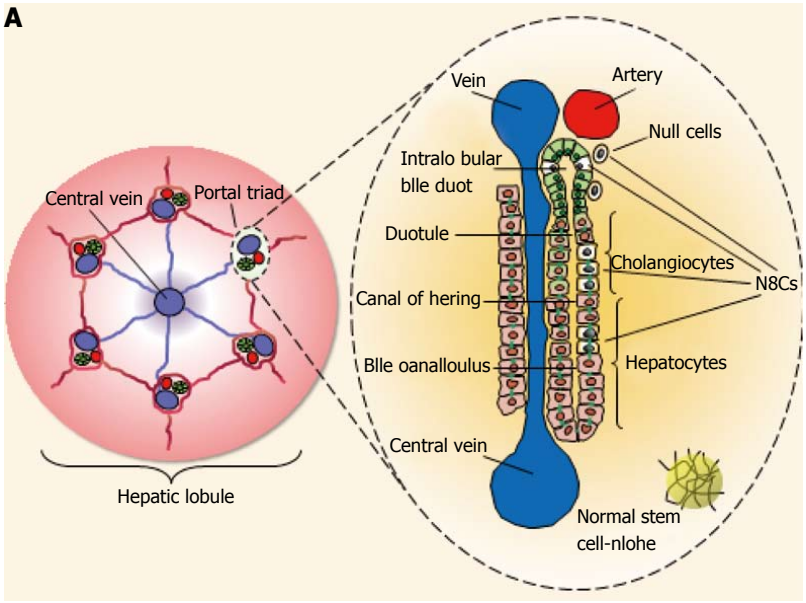
The TGF-β superfamily plays a key role in a wide range of cellular processes and is therefore tightly regulated. The pathway is both positively and negatively modulated, for example, by extracellular antagonists, ligand-binding antagonists, and the regulation of receptor function and inhibition by I-SMADs. Positive regulation plays a key role in amplifying signaling through the TGF-β superfamily[87]. Negative regulation could be critical for the restriction and termination of signaling. In order to prevent the type I receptor from becoming activated, BAMBI binds to it and serves as a negative regulator of the TGF-β signaling pathway. Similarly, FKBP12 can prevent phosphorylation by binding to the GS domain of type I receptors. The most important negative regulation in the signaling pathway is inhibition by I-SMAD, which plays a critical role in transduction of the TGF-β signaling pathway. I-SMAD has different ways of regulating signaling both in the cytoplasm and nucleus. SMAD7 has been proved to form a complex with the type I receptor to inhibit R-SMADs binding to the receptor and prevent their phosphorylation[88]. In the nucleus, SMAD6 binds to the transcription factor Hoxc-8 and acts as an antagonist of transduction. SMAD6 can also bind to DNA directly and recruit HDAC to inhibit transduction[89].

Non-SMAD signaling pathway

Besides the SMAD pathway, TGF-β also activates other signaling cascades. For example, TGF-β can activate TGF-β-kinase 1 (TAK 1), (ErK), p38, mitogen-activated protein kinase (MAPK) and Akt[90]. These non-canonical or non-SMAD dependent means of activating the TGF-β pathway appear to involve signaling *via* jun N-terminal kinase (JNK), p38 MAPK, ERK or MEKK. The TGF-β inhibitor, LY2109761, a surrogate compound of LY2157299, has also shown inhibitory activity towards the non-canonical pathway that includes FAK, β1 integrin, MEK, Erk, Akt, mTOR and PTEN, but not p-38-MAK-kinase[91]. In rat intestine or mink lung epithelial cells, rapid activation of p21 (Ras) occurred after TGF-β treatment, which indicated that TGF-β could activate the Erk-MAPK signaling pathway[92,93]. In this case, TGF-β induced Erk activation and tyrosine phosphorylation. Similarly, TGF-β can rapidly activate JNK through MKK4 and p38 MAPK through MKK3/6 in several cell lines[94]. In human prostate cancer cells, SMAD7 triggers apoptosis by an association with TAK1, MKK3 and p38 MAPK, to help activate the TAK1-p38 MAPK signaling pathway[90]. It is known that TGF-β is involved in activation of the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway, and recent studies also indicate that TGF-β utilizes the mTOR pathway to regulate cell survival, metabolism, migration, and invasion[95,96]. Moreover, TGF-β induced the activation or inactivation of small GTPases and the phosphorylation of Par6, which may be critical events leading to the EMT[97]. Both the SMAD and non-SMAD signaling pathways determine the end results of the cellular response to TGF-β.

ROLE OF TGF-α IN THE HCC MICROENVIRONMENT

In human HCC, cells with stem cell markers show a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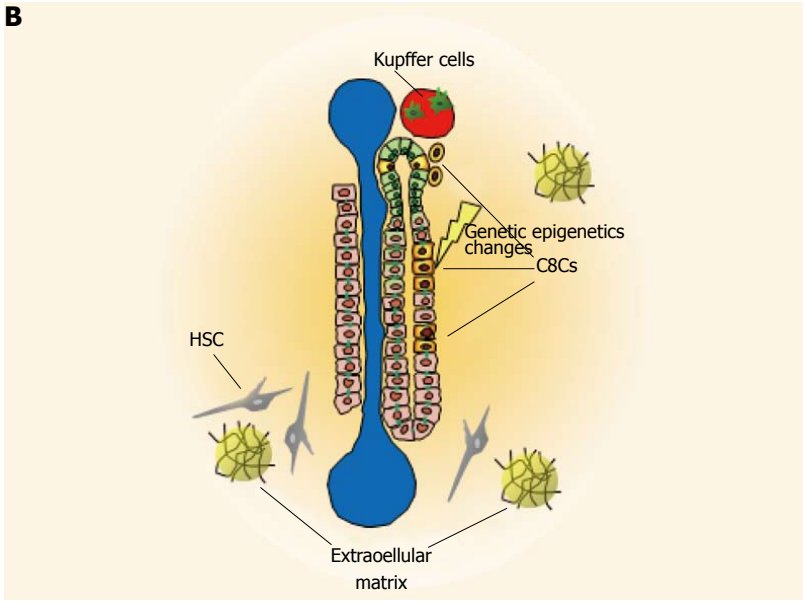
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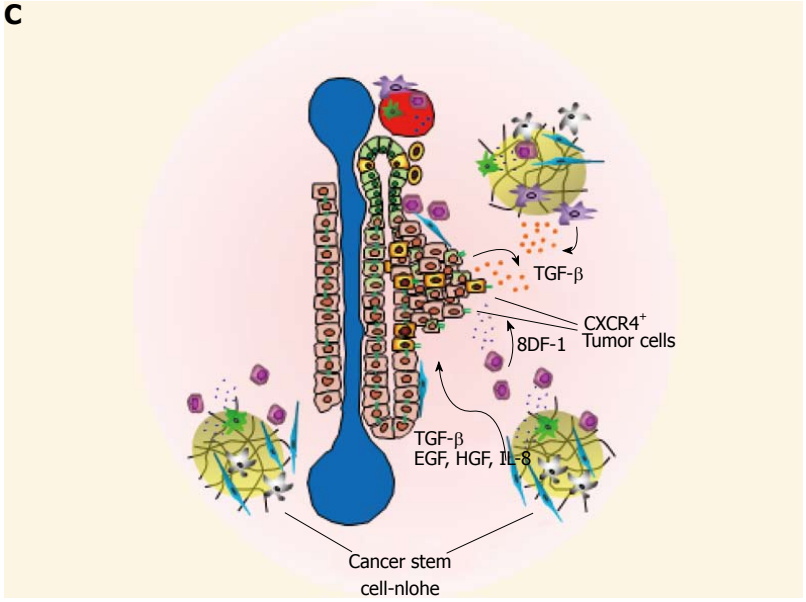
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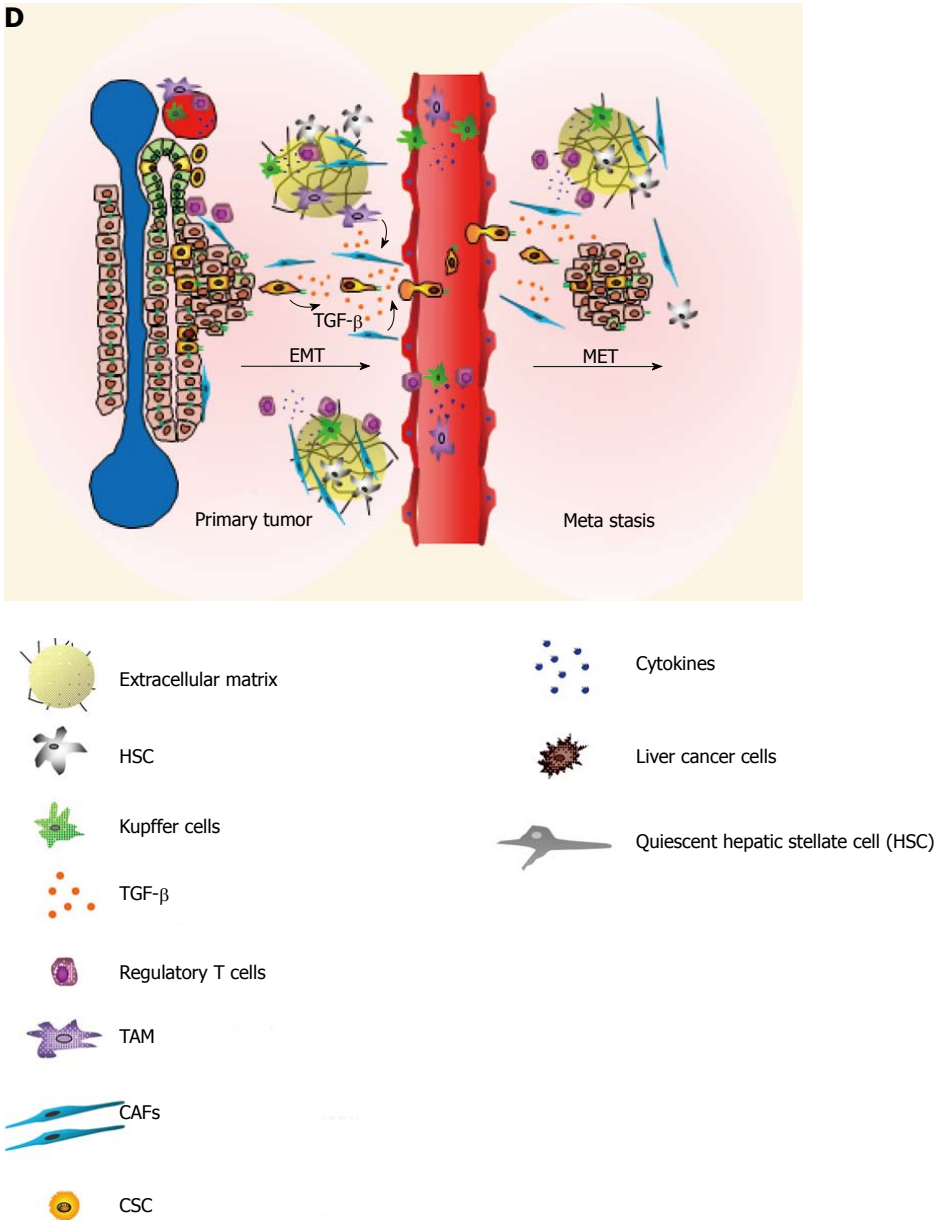

Figure 2 Role of the stem cell niche in liver tumors. A: A hepatic lobule consists of one central vein and six surrounding portal triads, each of which has bile ducts, a hepatic artery and portal vein. BrdU-retaining cells (with black nuclei) represent putative stem cells (NSC) and are located in cholangiocytes of the intralobular bile duct, together with less-characterized null cells, small hepatocytes at the interface of cholangiocytes, and hepatocytes in the canals of Hering; B: A series of genetic and epigenetic changes in NSC (or committed progenitor cells) leads to the generation of cancer stem cell (CSC) and then to expansion of cells within the stem cell niche. The niche adapts to the presence of CSCs by recruiting cells that would not normally be present; C: The signaling pathways for the activation, expansion and differentiation of stem/progenitor cells are good candidates for contributing, under suitable conditions, to a pro-tumorigenic role. Migration and metastasis of CSCs is a multistep process, in which SDF-1 plays a crucial role by chemoattracting CXCR4+ tumor cells; D: Transforming growth factor-β (TGF-β)-induced transdifferentiation of hepatocytes and liver tumor cells from an epithelial to a mesenchymal phenotype (EMT) increases a population of cells with putative liver progenitor properties. The EMT plays a fundamental role in tumor progression and metastasis and the TGF-β-induced EMT can guide cancer cells to delaminate from primary tumors, migrate along the extracellular matrix network, and arrive at the site of metastasis *via* the peripheral blood. HSC: Hepatic stellate cell; TAM: Tumor-associated macrophage; CAFs: Compressed air foam system; EGF: Endothelial growth factor; HGF: Hepatocyte growth factor; IL: Interleukin.

loss of TGF-β receptor Ⅱ and ELF (a β-spectrin that propagates TGF-β signal), and a marked activation of the IL-6 pathway, which is the major stem cell signaling pathway[98,99]. These data support the concept that the absence of TGF-β-driven epithelial differentiation favours carcinogenesis. Indeed, in addition to the well-known transcriptional responses predominantly addressing tumor suppressor actions, TGF-β induces other Smad-dependent or independent effects that contribute to tumor progression[100] (Figure 2C).

TGF-β induces trans-differentiation of hepatocytes and liver tumor cells from an epithelial to a mesenchymal phenotype, which results in a population of cells with putative liver progenitor properties[101,102]. Additionally, the TGF-β-induced EMT not only endows cells with migratory and invasive properties, but can also induce

cancer cells to dedifferentiate and gain cancer stem-cell-like properties[103]. Snail1 Snail2 and Twist may actively participate in this process[100,102,104] (Figure 2D). Furthermore, other studies have demonstrated the capacity of TGF-β to induce/maintain a stemness phenotype in other tumors[105].

CONCLUSION

In conclusion, several components of the tissue microenvironment have been shown to participate in modulating the biological behavior of HCC. Biological redundancy complicates the understanding of the tumor/host crosstalk. Nevertheless, we hypothesize that HCC should be considered as a tumor of the whole liver, rather than just of few hepatocytes that grow in nodules. Taking into account this scenario, new therapeutic approaches targeting the TGF-β pathway seem promising. Directed against the microenvironment, they might have the effect of making this microenvironment more hostile to HCC progression. Up to now, targeting TGF-β in HCC has been considered a questionable approach in view of the tumor suppressor role that TGF-β exerts on cells. This apparent controversy has been addressed by examining the differences between the early and late TGF-β signature in patients with HCC[106]. The clinical role of the late signature has been further confirmed in a wider HCC molecular classification, suggesting a potential role for TGF-β as a possible therapeutic target[107]. Currently, a multicenter clinical trial is ongoing, evaluating the efficacy and effectiveness of a TGF-β receptor I kinase inhibitor (LY2157299) in patients with HCC. However, it is not yet possible to predict at which stage of the disease such a drug would display the best therapeutic effect, as all the clinical trials so far have been restricted to patients with a more advanced disease stage of HCC, for ethical reasons. Nor is it yet possible to predict which patients might benefit from such treatment, according to the etiology, and how the underlying liver disease might interfere in the tumor/stroma crosstalk.

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ISSN 1007-9327
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