Tumor Microenvironment in Hepatocellular Carcinoma

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The interaction of the cellular environment surrounding the tumor plays a relevant role in hepatocellular carcinoma (HCC) pathogenesis. The tumor microenvironment is directly implicated in the modulation of liver fibrosis, the process of hepatocarcinogenesis, the epithelial-mesenchymal transition (EMT), invasion, and metastasis. Besides liver cancer cells, several cell types participate in the tumor progression in the liver.

Keywords: Hepatic Stellate Cells ; Fibroblasts ; Macrophages ; hepatocellular carcinoma ; tumor-associated macrophages (TAMs) ; Cancer-associated fibroblasts (CAFs)

1. Hepatic Stellate Cells

Hepatic stellate cells (HSCs) are major components of liver connective tissue. They are localized in the basolateral surface of hepatocytes and the anti-luminal side of sinusoidal cells [1]. HSCs are in charge of vitamin A storage, synthesis of matrix metalloproteinases (MMPs) and extracellular matrix components (ECM, collagen), release of cytokines (IL-6 and IL-1β), defensin-1, chemokines (CCL5, CCL2), and growth factors (TGF-α/β, EGF, PDGF, bFGF) [1],[2]. Normally, HSCs are in a quiescent state. Upon liver injury, they become activated, their cytoskeleton becomes remodeled through an increased expression of alpha-smooth muscle actin (α-SMA), and there is also a rise in cytokines, ECM components, and growth factors production [1]. In the activated state, HSCs transdifferentiate into myofibroblast-like cells. This phenotype makes them more contractile, so they can infiltrate the hepatocellular carcinoma (HCC) stroma and localize around fibrous septa, sinusoids, and capsules [3],[4].

Conditioned media from tumoral hepatocytes has been found to increase the proliferation of rat HSCs and induce the expression of HSCs' activation markers [5],[6]. Similarly, another study demonstrated that collected media from HSCs potentiated the tumorigenic capacity of HCC cancer cell lines [7]. The co-culture of hepatoma cells and activated HSCs also revealed the activation of genes related to inflammation, chemotaxis, angiogenesis, and metalloproteinase from microarray analysis data [8],[9]. Regarding in vivo studies, the co-implantation of HCC and HSCs cells in nude mice increased tumor growth via NF-κB and extracellular signal regulated kinase (ERK) pathways activation [7],[10]. In this sense, previous work has showed that angiogenin was responsible for the crosstalk between HCC and HSCs cells both in vitro and in mice models [11].

HSCs are also involved in the promotion of angiogenesis in HCC. Diverse mechanisms are responsible for this, among them the secretion of angiopoietin-1 [12] or IL-8 [13]. Moreover, PDGF secreted by tumor and endothelial cells has been described as attracting HSCs, while at the same time, HSCs secrete VEGF, thus promoting angiogenesis [14].

Several studies have pointed out that the secretion of IL-6 by HSCs may promote HCC progression [15],[16]. In an HCC murine model with obesity, insulin resistance, and dyslipidemia, fatty acid binding protein 4 (FABP4) was enriched in intra-tumoral HSCs, contributing to hepatocarcinogenesis [17]. The co-culture of HSCs with HCC cells demonstrated that the overexpression of miR-1246 secreted by HSCs or the silencing of its target RORα increased proliferation, invasion, and metastasis of HCC cells, with the involvement of the Wnt/β-catenin pathway [18].

HSCs have also been described as promoting tumor chemoresistance. The laminin-332/α3 integrin axis and the ubiquitination of focal adhesion kinase (FAK) by HSCs were demonstrated to be involved in sorafenib chemoresistance [19]. In addition, FGF9, expressed only by HSCs, promoted the tumorigenic capacity of HCC cells and the resistance to sorafenib, and FGF9 overexpression was associated with poor prognosis in patients with HCC [20]. Interestingly, the tyrosine kinase AXL, interesting target in cancer therapy, is increased in activated HSCs [21] and in dietary models of liver fibrosis [22].

2. Cancer-Associated Fibroblasts

Fibroblasts are present in the fibrillar matrix of connective tissue. They are responsible for wound healing, formation of extracellular matrix (ECM), tissue maturation, and the inflammatory response [23]. Cancer-associated fibroblasts (CAFs) are a sub-group of fibroblasts that are activated and implicated in cancer progression. Although CAFs arise from normal fibroblasts, CAFs can also derive from epithelial cells, endothelial cells, smooth muscle cells, bone marrow-derived progenitor cells, and pre-adipocytes [24]. Additionally, HCC tumors frequently develop in a cirrhotic liver in which there is a great amount of activated fibroblasts [25]. Therefore, CAFs may contribute to HCC tumor progression by producing growth factors (EGF, FGF, HGF, and TGF-β), chemokines (SDF-1), cytokines (IL-6), and metalloproteinases (MMP-3 and MMP-9) [26],[27],[28]. In addition, there is a growing amount of evidence showing that the crosstalk between CAFs and HCC tumors could be mediated by miRNAs contained in exosomes. For example, low miR-150-3p levels secreted by CAFs have been discovered to be involved in HCC migration and invasiveness as well as poor clinical outcome [29]. Interestingly, the upregulation of mirR-335-5p by CAFs inhibited HCC tumor cells proliferation in vitro and in vivo [30]. Moreover, HCC tumor cells were found to induce the conversion of HSCs into CAFs through the secretion of miR-21, which promoted cancer progression via the secretion of the angiogenic factors VEGF, MMP2, MMP9, bFGF and TGF-β [31].

3. Tumor-Associated Macrophages

Macrophages around the tumor site are called tumor-associated macrophages (TAMs). Macrophages can display the M1 (classic) or M2 (alternative) phenotype depending on their tumor-suppressing or tumor-promoting role [32]. M1 macrophages produce Th1-cytokines, such as IFN-γ, and are activated by LPS and other microbial antigens. They exhibit high antigen-presenting capacity and increased cytotoxic activity, thereby producing reactive oxygen species (ROS) [33]. On the contrary, M2 macrophages are polarized by Th2-type cytokines IL-4, IL-13, glucocorticoids, and TGF-β. Their antigen-presenting capacity is low. M2 macrophages decrease inflammation, suppress the adaptive immune system, and promote tumor progression, angiogenesis, and tissue repair [34].

In HCC, M2 macrophages have been found to promote tumor progression and metastasis with the involvement of glypican-3, a member of the glypican family of heparin-sulfate proteoglycans reported to be highly expressed in the majority (>70%) of HCCs [35]. In addition, TGF-β1 secretion by TAMs promoted cancer progression and EMT in HCC [36], [37], and moreover, the TAM-production of IL-6, via STAT3, also promoted stemness in HCC [38]. Moreover, in a murine model of HCC, intra-tumoral macrophages expressing MMP-9 were involved in ECM remodeling, thus favoring tumor progression [39], while, in another study, the presence of TAMs correlated with tumor vascularity, pointing towards the ability of TAMs to promote angiogenesis [40].

It has been shown, in Hepa1-6 HCC tumors, that, in the early phase of tumor development, infiltrated macrophages displayed a tumor-suppressing phenotype, while, at advanced stages, the TAM population increases and is associated with tumor progression [41]. Thus, it is becoming apparent that macrophage polarization plays a crucial role in the initiation of liver diseases, and its role in HCC needs to be further clarified, particularly since it may affect immunotherapy efficacy [42]. At the same time, tumor cells have been found to release Wnt ligands that promoted M2 polarization of macrophages and, in turn, promoted tumor growth, invasion, and immunosuppression in HCC [43]. In this regard, treatment of HCC with sorafenib has been shown to induce the repolarization of alternative macrophages to M1 phenotype through IGF-1 signaling [44].

Additionally, in HCC human samples, TAM infiltration was linked with PD-L1 overexpression [45]. Although M1 macrophages have been considered to exert an anti-tumor role, M1 macrophages my promote PD-L1 expression in HCC tumor cells, highlighting the potential role of M1 macrophages in tumor promotion through IL-1β pathway [46]. In fact, Kupffer cells, resident macrophages in the liver, have been reported to mediate tumor growth in HCC by producing PD-L1 that interacts with PD-1 receptor in $CD8^+$ T cells, impairing $CD8^+$ T cell response [47]. In addition, Kupffer cells produce osteopontin, which is involved in inflammation, tumor progression, and metastasis [48].

4. Endothelial Cells

Endothelial cells (ECs) are present in the interior face of blood vessels. Other cells, such as HSCs, participate in controlling the size and elasticity of liver vessels [49]. The interactions of ECs with the ECM and basement membrane proteins play a role in proliferation, stability, and neoangiogenesis. When the basement membrane degrades, ECs become exposed to collagen, which triggers the formation of new blood vessels [50]. Neovascularization favors tumor proliferation, invasion, and metastasis, since the new blood supply provides oxygen and nutrients to the tumor [51]. Tumor blood vessels have structural abnormality and increased permeability. ECs carry angiogenic receptors, for instance

VEGFR, EGFR, PDGFR, and CXCR [52]. Additionally, hypoxia is a known driver of tumor angiogenesis. Many studies conducted in HCC preclinical models have shown that hypoxia-inducible factor (HIF) proteins led to the activation of VEGF, which promotes angiogenesis [53],[54],[55]. VEGF and VEGFRs are crucial for HCC development [56],[57]. The binding of VEGF ligands to their receptors elicits downstream phosphorylation that results in EC proliferation and the formation of new branches of blood vessels [58]. High VEGF levels in serum have been found to be associated with bad prognosis in HCC patients who underwent surgical resection [59], since sVEGF concentration has been showed to correlate with angiogenesis, invasion, and metastasis of HCC [60]. The interaction of platelet-derived growth factors (PDGF) with PDGF receptors (PDGFR) triggers the activation of the same signaling pathways as the binding of VEGF and VEGFRs not only in ECs but also in fibroblasts, smooth muscle cells, and HSCs [61]. In this sense, PDGFRα expression was associated with microvascular invasion [62].

Additionally, fibroblast growth factor (FGF) and fibroblast growth factor receptors (FGFR) also regulate cell growth and angiogenesis [63]. Basic fibroblast growth factor (bFGF) fostered VEGF expression and its synergistic effect contributed to HCC development and neovascularization [64]. Of interest, angiopoietin-1 (Ang-1) and 2 (Ang-2) bind to their receptor, Tie2, to stimulate angiogenesis [65]. Ang-1 and Ang-2 expression was detected in hepatoma, HSCs, ECs, and smooth muscle cells, while Tie2 receptor was only identified in ECs, HSCs, smooth muscle cells, and monocytes [66],[67]. Ang-2 serum levels were high in patients with cirrhosis and HCC [68], being a prognosis marker [69]. Ang-2 exhibited a synergistic effect with VEGF in the development of angiogenesis in HCC in mice through the activation of MMP-2 and MMP-9 [70]. Ang-2 was included in a five-gene signature that effectively predicted HCC rapid growth [71]. As other pro-angiogenic factors, Ang-2 also played a role in the promotion of HCC invasion and metastasis [72].

5. Tumor-Associated Cells of the Innate Immune System

Innate immune mechanisms may support or neutralize tumor-related immune activation, being recognized drivers of disease progression in the liver, particularly during conditions such as fibrosis or cirrhosis prior to HCC. Exhaustive research has been developed to delineate the immunological steps involved in the initiation and evolution of liver cancer. During HCC progression, several studies analyzing the response to immunotherapy have leaded to conflicting results, probably due to the complex and only partially known interactions between specific immune cells, tumor cells, and the different cells that configure the tumor microenvironment. For reviews on the subject, see [73],[74].

Forgotten during years, tumor-infiltrating immune cells in the HCC have been recently evaluated and characterized [74]. For many solid tumors, including HCC, different relationships between immune cell populations and therapy efficacy and prognosis have been suggested. While the complete impact of the tumor immune environment is still to be determined, myeloid cells including TAMs and myeloid-derived suppressor cells (MDSCs) are abundantly present in the HCC microenvironment being frequently associated with poor prognosis. In general, myeloid cells in HCC play a very active role in promoting tumor initiation, development, angiogenesis, metastasis, and even therapeutic resistance [75]. In contrast, increasing numbers of infiltrating T-effector cells are habitually linked with a good prognosis [76]. Generally, a pro-inflammatory HCC ambient with infiltrating natural killer (NK) cells, and CD8-expressing T cells are considered to be positive and associated with good clinical outcomes in numerous tumor types [77]. NK cells play a central role in hepatic immunity, accounting for 25–50% of the total number of liver lymphocytes. Both circulating and tumor infiltrating NK cells are positively correlated with patient survival benefit in HCC [78], contrary to other immune cells, such as MDSCs and regulatory T cells, which seem to disrupt the immune control of the HCC [76].

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