

# Importance of tumor/stroma interactions in prognosis of hepatocellular carcinoma

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**Abstract:** Hepatocellular carcinoma (HCC) is the third largest cause of cancer deaths worldwide. It seems to be needed to find new ways to address the mechanisms involved in the progression of HCC, which can provide a prognostic evaluation and new therapeutic targets. Several studies have established that crosstalk between tumor cells and the microenvironment plays a key role in tumor progression and metastasis. In this context, the work of Zhu *et al.* contributes to assess interactions between tumor and microenvironment associated-macrophages promoting tumor progression and metastasis. Indeed, they concluded that the interplay of osteopontin (OPN) and peritumoral macrophages (PTMs) represents a new insight into tumor progression and therapeutic targets for HCC. Historically, tumor-infiltrating leukocytes have been considered to be manifestations of an intrinsic defensive mechanism against developing tumors, however, now, it is known that leukocytes infiltration can promote tumor phenotypes, such as angiogenesis, growth, and invasion. Characterization of functional heterogeneity of stromal cell components, and specifically the analysis of stromal fibroblasts can provide a new focus on mechanisms involved in the progression of HCC. All of this opens the possibility to provide prognostic information for HCC based on biological parameters derived from peritumoral status from tumors.

**Keywords:** Hepatocellular carcinoma (HCC); tumor microenvironment; macrophages; fibroblasts

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Hepatocellular carcinoma (HCC) is the third largest cause of cancer deaths worldwide. The 5-year risk of HCC recurrence after resection is as high as 70% because the underlying chronic liver disease continues to put the patient at risk for the development of a new one (1). Even in those patients with early-stage disease, tumor relapse after treatment remains the major obstacle for outcomes improvement. Recent advances in whole-genome technologies have revealed an overwhelming amount of molecular data on human carcinomas, including HCC. However, spite of all of these data, HCC prognostic evaluation is based on clinicopathological parameters such as tumor stage. This reflects the complexity and heterogeneity of HCC biology, and it leads us to consider the need to find new ways to address the mechanisms involved in the progression of HCC, which can provide a

prognostic evaluation and new therapeutic targets.

There are several evidences indicating that progression of solid tumors towards a malignant phenotype does not depend exclusively on cell-autonomous properties of cancer cells, but is also deeply influenced by tumor stroma reactivity (2). Crosstalk between tumor cells and the microenvironment plays a key role in tumor progression and metastasis. The two well-studied and populous cellular component of the tumor stroma are mononuclear inflammatory cells (MICs) and cancer associated fibroblasts (CAFs). On the basis of this concept, recently Zhu *et al.* (3) have attempted to elucidate the prognostic significance of combining tumor-secreted osteopontin (OPN) with microenvironment-associated peritumoral macrophages (PTMs) in HCC, especially for those with early-stage disease. Using tissue microarray-based

immunohistochemistry, they have investigated OPN and PTMs expression in two independent cohorts consisting of 374 patients with HCC who underwent radical resection. The prognostic value of these two factors, alone or in combination, was investigated in these patients. They found that OPN combined with PTMs was a significant and independent prognostic factor for both overall survival and time to recurrence from the learning cohort (n=96). Their combined value for prognosis was validated in early-stage HCCs using another independent cohort. This combination remained significant in HCCs with low  $\alpha$ -fetoprotein levels in both cohorts, and was predictive for early recurrence and death risk (<2 years) compared with a single marker. Therefore, Zhu *et al.* (3) have concluded that tumor OPN combined with PTMs is a promising predictor of tumor recurrence and survival in patients with HCC, especially for those with early-stage disease, and that the interplay of OPN and PTMs represents a new insight into tumor progression and therapeutic targets for HCC.

These results support previous studies reporting a key role of the stroma in tumor progression. Tumors are composed not only of cancer cells but also of other cell types constituting the stroma. These stromal cells include CAFs, endothelial cells, pericytes, and a variable representation of leukocytes. Leukocytes can represent up to 50% of the total tumor mass in many human tumors. Initially, tumor cells and tumor microenvironment, respond to tumor hypoxia and necrosis secondary to excessive tumor cell proliferation, by releasing a number of growth factors and cytokines that are chemoattractive for monocytes and macrophages [colony stimulating factor (CSF)-1, granulocyte-macrophage (GM)-CSF, transforming growth factor (TGF)- $\beta$  and chemokines] (4). In this context, the work of Zhu *et al.* contributes to assess interactions between tumor and microenvironment associated-macrophages facilitating tumor progression and metastasis. This is due to that OPN has alternatively been suggested as a possible stimulator of immune function, a chemoattractant for macrophages and endothelial cells, and a tumor defense against cytotoxic macrophages (5). The authors hypothesized that high OPN expression in tumor tissues could recruit more macrophages in the peritumoral liver tissue and facilitates HCC growth and metastasis, resulting in a dismal survival rate. In addition, it is relevant to note that the peritumoral invasive front is the area where some of the most important interactions between cancer cells and tumor supporting stroma take place.

Historically, tumor-infiltrating leukocytes have been considered to be manifestations of an intrinsic defensive

mechanism against developing tumors. The presence of leukocytes in tumors was subsequently interpreted as an aborted attempt of the immune system to reject the tumor. However, increasing evidence indicates that leukocytes infiltration can promote tumor phenotypes, such as angiogenesis, growth, and invasion. This may be due inflammatory cells probably influence cancer promotion by secreting cytokines, growth factors, chemokines and proteases, which stimulate proliferation and invasiveness of cancer cells (6). Indeed, accumulating clinical data for solid tumors show a correlation between high-density leukocytic infiltration into tumors and poor outcome of patients with several malignancies of very different origins (such as of breast, bladder, rectum, endometrium, melanomas, gliomas or leiomyosarcomas). Nevertheless, the presence of inflammatory cells can be an indicators of favorable prognosis in some tumor types, as for example the presence of macrophages in colorectal cancer, gastric or ovarian carcinomas (6). The controversy over the prognostic significance of lymphoid infiltrate in the tumor site, may be due to the fact that the criteria for evaluation of tumor infiltrates are not sufficiently standardized to produce reliable and reproducible results in different institutions. Leukocyte infiltrate includes a variable representation of leukocytes, including macrophages, neutrophils, mast cells, and T and B lymphocytes. In addition, inflammatory cells and immunomodulatory mediators present in the tumor microenvironment polarize host immune response toward specific phenotypes impacting tumor progression.

Such as mentioned Zhu *et al.* in their work, the macrophage is a pivotal member in tumor stroma, strongly correlated with poor prognosis in different types of solid tumors, including HCC (7,8). Macrophages are often the most abundant immune cells population in the tumor microenvironment. It has been reported that, once recruited to tumors, macrophages can assume two different phenotypes: M1 or M2, based on environmental stimuli and each expressing specialized functional properties (9). The M1 phenotype is associated with inflammation and microbial killing activity, whereas M2 phenotype is associated with activities which are predominant and key events in cancer, including inhibition of Th1 adaptive immunity by immunosuppressive mediators (TGF $\beta$ , IL-10 or PGE2), production of growth and survival factors (EGF, IL-6 and CXCL8), secretion of angiogenic factors (VEGF, TGF $\alpha$  or PGE2), production of matrix metalloproteases (MMPs) which degrade extracellular matrix (ECM), and chemokines capable of recruiting more inflammatory cells

(CCL17, CCL18 or CCL22) (9).

Results obtained by Zhu *et al.* suggest other further and interesting lines of investigation in HCC. One of these may be to investigate the clinical relevance of the relative amount of macrophages (CD68<sup>+</sup>), T-cells (CD3<sup>+</sup>) and B-cells (CD20<sup>+</sup>). It is known that macrophages have several pro-tumor functions and their infiltration into the tumor has been associated with worse prognosis (10-12). By contrary, it has been reported that both T- and B-lymphocytes perform an important immunological response by inhibiting cancer development and progression (13,14). In this line, for example recently we found that an increased CD68 count and CD68/(CD3<sup>+</sup>CD20) ratio in the invasive front were directly associated with a higher probability of shortened relapse-free survival in breast cancer (15).

An important aspect in studies of the mechanisms involved in the progression of HCC, is the characterization of functional heterogeneity of stromal cell components, and specifically the analysis of stromal fibroblasts. Normal fibroblasts are the most abundant cell type in the connective tissue and are responsible for the synthesis and turnover of the ECM. However, characteristics of CAFs are distinct from that of normal fibroblasts, including a higher proliferation rate, as well as capacity to promote tumor phenotypes such as survival, proliferation, metabolism reprogramming, angiogenic shift, ECM remodeling, EMT activation, stem cell trait achievement, metabolic reprogramming toward a reverse Warburg phenotype, or inflammatory cells recruitment (16). All of characteristics of CAFs may be due to the production of a repertoire of growth factors and cytokines that influence the behavior of the epithelium, such as HGF, EGF, IGFs, IGFs, b-FGF or TGF $\beta$  (2). It is also known that CAFs are capable of evoking a proinflammatory response. After activation, CAFs initiate a pro-inflammatory response including the secretion of IL-1 $\beta$ , IL-6, IL-8, SDF-1, and nuclear factor kappa B (NF- $\kappa$ B), which may induce inflammation by recruiting components of the immune system (16). Thus, CAFs may orchestrate a distorted architecture of the host tissue and a functional "corrupted" stroma which in turn helps metastatic spread. In accordance with this, it was of note our findings indicating that the expression of several metalloproteases and their inhibitors, which are implicated in invasion and metastasis, by fibroblasts or MICs was associated with a poor prognostic in HCC (17). Likewise, TLR9 expression by fibroblast-like cells was significantly associated with a shortened overall survival in patients with HCC (18). Although strong pro-inflammatory and antiviral responses after TLR stimulation may be beneficial in the short term to eradicate pathogens, a prolonged or

exaggerated activation of TLR signaling may have deleterious effects. As molecular sensors, TLRs detect pathogen-derived products and couple to different adapter proteins that trigger specific signaling pathways such as the interleukin 1 (IL1) receptor-associated kinase (IRAK) family and TANK-binding kinase 1 (TBK-1). These adapters initiate pathways leading to the activation of their respective transcription factors, NF- $\kappa$ B and interferon regulatory factor 3 (IRF3), which induce the release of various immune and inflammatory cytokines [such as tumor necrosis factor (TNF) and IL6] and carcinogenesis (19).

The liver is probably a good example for the link between chronic inflammation and cancer that was postulated by Rudolf Virchow more than 100 years ago (20). It is estimated that almost 80% of HCC in the western world develop as a consequence of chronic inflammation and arise in fibrotic or cirrhotic livers. Due to its anatomical links to the gut, the liver is constantly exposed, via the portal vein, to gut-derived bacterial products, viral infection, alcohol or other products, which may be cause of chronic liver damage, therefore increasing risk for HCC. Now, the paper of Zhu *et al.*, open the possibility to provide prognostic information for HCC based on biological parameters derived from inflammatory peritumoral status from tumors.

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