# Tumor-Associated Macrophages in the Progression of Hepatocellular Carcinoma

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***Abstract:*** Hepatocellular carcinoma (HCC) contains many immune cell matrices, which constitute the tumor microenvironment (TME). Tumor-associated macrophages (TAM) are the main compartments of immune cell matrix in HCC, which play an important role in the pathogenesis of HCC, including immunosuppression, angiogenesis, tumor invasion and metastasis, and malignant transformation of HCC stem cells. At present, targeted TAM therapy for HCC has achieved promising results by eliminating existing TAMs, blocking the recruitment of TAMs, reprogramming TAMs polarization, regulating TAMs products and restoring TAMs phagocytosis. This review summarizes our understanding of TAMs and HCC, and discusses the role of TAMs in the development of HCC.

***Keywords:*** Tumor-Associated Macrophages; Immunotherapy; Hepatocellular Carcinoma

## 1. Introduction

Liver cancer is the fourth most common cause of cancer-related death worldwide, and its incidence is on the rise worldwide[1] . HCC is the most common type of liver cancer, accounting for about 90% of all cases. Compared with other common solid tumors, the prognosis of patients with HCC was poor, and the overall 5-year survival rate was 18%. Patients with early and medium-term HCC can receive a variety of effective treatments, including surgical hepatectomy, liver transplantation, ablation and transcatheter arterial chemoembolization.However, The main treatment for advanced HCC is systematic therapy, including tyrosine kinase inhibitors sorafenib, lamvatinib and immune checkpoint blockers (ICBS) [2].However, suppression of immune checkpoints with anti-EGFR antibodies atrizumab and bevacizumab has become a first-line treatment for patients with advanced liver cancer [2] . TME consists of many components that coexist and interact with each other, including tumor-associated macrophages, CD4 and CD8 T cells, dendritic cells (DC), natural killer (NK) cells, tumor-associated endothelial cells (EC), abnormal tumor vascular system, cancer-associated fibroblasts (CAF) and myelogenic immunosuppressive cells (MDSC). TME is a dynamic environment coordinated by multiple cells and non-cells, and each component or component hopefully represents a potential indicator of re-editing TME [3] . However, as one of the most abundant immune cells in infiltrating TME, TAM exists in all stages of liver cancer progression.

As far as we know, the interaction, potential mechanism and therapeutic effect of TAMs on HCC regulation have not been fully understood and rarely reported. In this review, we aim to provide the latest and comprehensive updates on TAM in HCC. And focuses on the role of TAMs in HCC, to provide a solid theoretical basis for the discovery of related targeted drugs.

## 2. Activation and activation of TAM in microenvironment of hepatocellular carcinoma

TAM is recruited and activated by different chemokines in the TME of HCC and differentiates into specific polarization forms related to specific pathological conditions. According to the state and function of macrophage activation, macrophages can be divided into two different polarization states: classical activated M1 macrophages and alternately activated M2 macrophages[4]. The two forms of polarization are interchangeable under certain circumstances and in the presence of certain stimuli. M1 macrophages usually play a pro-inflammatory role and secrete a large number of pro-inflammatory cytokines. Classical macrophage activation occurs when cells are stimulated by:1) lipopolysaccharide, a component of the cell wall of Gram-negative bacteria; 2) IFN- γ released by NK cells and type 1 T helper cells (Th1);3) tumor necrosis factor (TNF); 4) granulocyte macrophage colony stimulating factor (GM-CSF) ; 5) Toll-like receptor (TLR) ligands [5] . Activated M1 macrophages secrete some interleukines, chemokines and TNF- α to induce pro-inflammatory effects.

In contrast, M2 macrophages usually perform the opposite function as M1 macrophages. Cytokines IL-4, IL-10, IL-13 and transforming growth factor β (TGF- β) are secreted by Th2 cells and tumor cells. CSF1 and prostaglandin E2 (PGE2) can induce alternative activation of macrophages and lead to M2 polarization phenotype. M2 macrophages can secrete several complex immunosuppressive factors, cytokines and growth factors, regulate Th-2 immune response, promote tumor cell growth and participate in tumor angiogenesis [6] .

## 3. M1-TAM and liver cancer

Classical activated macrophages show anticancer properties. In HCC, M1 can inhibit tumor progression through various mechanisms. Expression of sirtuin1 in hepatoma cells regulates M1 polarization through NF- κB pathway[7].In addition, monocytes overexpressing IL-12 can down-regulate phosphorylated signal transducers and activators of transcription 3 (p-STAT-3) and c-Myc to differentiate into M1 and inhibit HCC growth. However, M1 also shows a positive correlation with cancer, and this abnormal mechanism is relatively rare. For example, M1 macrophages secrete IL-1 β to activate hepatoma cells and induce PD-L1 expression through transcription factors IRF1 and NF- κB [8] . Therefore, M1-TAM and M2-TAM are not always mutually exclusive; On the contrary, these two types of cells often coexist in TME. So these two types of macrophages cannot be considered to be completely different macrophage populations. The preferred function of mixed TAMs phenotype depends on the balance between macrophage activation and inhibition and immune microenvironment.

## 4. M2-TAM and HCC

## 4.1 M2-TAM promotes proliferation, invasiveness and metastasis of cancer cells

Although most studies have focused on tumor-derived exosomes, the existence of TAM-derived exosomes is necessary for tumor progression and metastasis. Alternatively, activated (M2) macrophages can secrete cytokines CCL2 to enhance tumor invasion and induce epithelial mesenchymal transformation (EMT) through Smad2/3 and Smad1/5/8 activation and snail upregulation[9] . In addition, CCL2 secreted by M1 macrophages is closely related to tumor dryness and EMT in TGF-β 1 and Wnt/ β catenin signaling pathways [10]. In vitro data show that M2-TAMs coordinates the immune microenvironment of iCCA by secreting various cytokines (such as TNF- α, ICAM-1,IL-6) and regulating EMT of cancer cells [10] . Interestingly, enhanced communication between TAM and tumor-related cells also promotes cancer invasion and metastasis.

The above studies show that macrophages play an exciting role in tumorigenesis. Therefore, an attractive therapeutic strategy for HCC may be to block the communication between M2-TAM and HCC cells, such as the use of non-coding RNA inhibitors and TAMs receptor inhibitors.

## 4.2 M2 TAM stimulates angiogenesis of HCC

Because of the angiogenic nature of most HCC tumors, angiogenesis is very important for the occurrence and metastasis of HCC. Angiogenesis in HCC is a multidimensional process coordinated by HCC cells and a series of tumor-associated stromal cells, including TAM and its bioactive products. In recent years, monocyte / macrophage subsets characterized by tyrosine kinase receptor Tie-2 expression have attracted much attention. TEM is mainly concentrated in the perivascular area of tumor tissue and participates in HCC angiogenesis.Macrophage population and phenotype were positively correlated with angiogenesis and clinical prognosis of HCC. CCR+2TAM is more abundant in the margin of highly vascularized HCC, while the lack of CCR2TAM infiltration can reduce pathogenic vascularization [11] . A case-control study showed that CD14 inflammatory macrophages secrete a large amount of IL-23 after stimulation of hepatitis virus-infected hepatocytes, accompanied by up-regulation of IL-23 receptors and strong macrophage-associated angiogenesis [12].

The angiogenic characteristics of TAMs and the angiogenic mimics in TME are the root causes of poor prognosis of tumor patients. In the preclinical model, the accumulation of macrophages is related to the emergence of drug resistance to anti-VEGF therapy [13]. The escape of VEGF targeted therapy may be due to the down-regulation of VEGFR-1 and VEGFR-3 and the up-regulation of angiogenesis-promoting genes. This key finding suggests that the use of VEGF blockers in combination with macrophage blockers, such as CSF1 or CCR2 inhibitors, can enhance the anti-VEGF therapeutic response.

# 5. Future and prospect

Although the preclinical and clinical studies of TAM provide encouraging results, there are still some challenges in using macrophages as targeted therapy for liver cancer. First, most TAM studies are limited to animal models. There is considerable heterogeneity between mouse model and human in pathogenesis and response to drug treatment. Secondly, due to the diversity of the origin of macrophages and the heterogeneity after differentiation, TAM shows different characteristics in different stages. It seems that the use of specific blockers is not sufficient to overcome liver malignant tumors,such as CSF1 blocking for all macrophages, resulting in systemic toxicity. Third, checkpoint blocking on the surface of macrophages is currently limited to PD-L1. Several novel immune checkpoints are expressed on the surface of macrophages, such as SIRP α and Tim-3. Finally, the elimination of TAM seems to lead to the compensatory appearance of other immunosuppressive cells. Therefore, TAM elimination also requires compensation for other immunosuppressive cells, such as Tregs and MDSC, which are only resistant to targeted TAM.

This article describes the origin of TAMs, the communication between TAMs and surrounding cells and the latest progress in treatment, which provides more options and substantial evidence for targeted macrophage therapy for patients with HCC. In the future, drugs targeting macrophages in the specific immune environment of the liver and more stable, safe and efficient immunotherapy can promote the further development of immunotherapy for HCC.

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