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## The mononuclear phagocyte system in hepatocellular carcinoma

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### <sup>1</sup> Abstract:

The mononuclear phagocyte system (MPS) consists of monocytes, dendritic cells (DCs) and macrophages, which play vital roles in innate immune defense against cancer. Hepatocellular carcinoma (HCC) is a complex disease that is affected or initiated by many factors, including chronic hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, metabolic disorders or alcohol consumption. Liver function, tumor stage and the performance status of patients affect HCC clinical outcomes. According to studies, targeted treatment of tumor microenvironment disorders may improve the efficacy of HCC treatments. Cytokines derived from the innate immune response can regulate T-cell differentiation, thereby shaping adaptive immunity, which is associated with the prognosis of HCC. Therefore, it is important to illustrate the function of the MPS in the progression of HCC. In this review, we outline the impact of HCC on the MPS. We illustrate how HCC reshapes MPS cell phenotype remodeling and the production of associated cytokines and characterize the function and impairment of the MPS in HCC.

Key words: Mononuclear phagocyte system; Hepatocellular carcinoma; Monocyte; Dendritic cell; Macrophage

### <sup>17</sup> INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is predicted to be the sixth most-diagnosed cancer and the fourth-leading cause of death among all types of cancers[1-3]. HCC is affected or initiated by many factors, including chronic hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, metabolic disorder or chronic alcohol

consumption[4-7]. More than 85% of HCC cases are accompanied by HBV infection in China[8-9]. Evidence has shown that HCC is a type of tumor with low or moderate immunogenicity[10]. Chronic inflammation creates an immunosuppressive microenvironment in the liver, facilitating HCC tumorigenesis and progression[11]. Although great progress has been made in the treatment of HCC in recent decades, the long-term survival rate of HCC is still poor[12]. Many factors, such as liver function, tumor stage and the performance status of patients, affect HCC clinical outcomes. According to previous studies, targeted treatment of disordered tumor microenvironments may improve the efficacy of HCC treatments[11,12].

The mononuclear phagocyte system (MPS) includes monocytes, dendritic cells (DCs) and macrophages, which was proposed by Van Furth in the late 1960s and early 1970s[13,14]. Monocytes, DCs and macrophages are important members of the innate immune system and are capable of modulating adaptive immune responses. The immune system is precisely balanced between immune activation and tolerance. Within this system antigen presenting cells (APCs) play a critical role in orchestrating the immune response[15]. Although stellate cells, endothelial cells and hepatocytes in the liver have the potential to present antigens to T cells, the MPS play a major role in determining the nature of the immune response and keep liver as a immune tolerance organ. MPS appear to be superior at sampling their environment through phagocytosis, and presenting antigen to T cells via MHC class II molecules[16]. APCs of the MPS appear to have a commensurately increased expression of antigen presentation and co-stimulatory molecules, and are potent secretors of modulatory cytokines[17].

Besides the role in tissue development, homeostasis, inflammation and innate immune defense against pathogens, the MPS also plays a vital role in cancers[17,18]. Innate immune cell-derived cytokines can regulate T-cell differentiation, thereby shaping adaptive immunity, which is associated with the prognosis of HCC. It is important to illustrate the important role of the

MPS in the progression of HCC. We discuss how the HCC microenvironment remodels MPS cell phenotypes and cytokine production and describe the function and impairment of MPS components in HCC in this review.

## **IMPACT OF THE MPS ON HCC**

### ***Monocytes and HCC***

Monocytes originate in the bone marrow and spleen and account for approximately 5%-10% of human peripheral blood mononuclear cells[13]. Monocytes transform into macrophages and DCs during inflammation[19-23]. It has been reported that the increase in activated monocytes (HLA-DR<sup>high</sup>CD68<sup>+</sup> cells) in the liver is related to disease progression[24]. Chemokine (C-C motif) ligand 15 (CCL15) recruits CCR1+CD14<sup>+</sup> monocytes to the edge of HCC tissue. High expression of CCL15 is associated with poor clinical prognosis. CCR1+CD14<sup>+</sup> monocytes suppress antitumor immunity, facilitate tumor metastasis and promote tumor cell proliferation and invasion[25]. Blocking CCL2/CCR2-mediated macrophage accumulation has been proposed as a treatment strategy for HCC[26-29].

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid progenitors and immature myeloid cells[30]. MDSCs in the tumor microenvironment, which are essential for tumor progression and are effective inhibitors of NK cells in HCC patients, play an important role in immunosuppression[31-33]. In humans, MDSCs usually express CD11b and CD33 and have low or no expression of HLA-DR. Monocyte-derived MDSCs (mMDSCs) express CD14, while granulocyte-derived MDSCs named (gMDSCs) express CD15 and CD66b[34]. MDSCs inhibit T cell and NK cell proliferation and have an inhibitory effect on conventional NK cells in a TIGIT-dependent manner. Compared with conventional NK cells, adaptive NK cells express lower levels of TIGIT and resist the inhibitory effect of MDSCs[32]. MDSCs express CD155, which is induced by ROS, and inhibit NK cell function via the CD155-TIGIT interaction[35]. Compared with normal liver tissues, hepatoma tissues are rich in granulocytes and mononuclear cells.

Mononuclear cells and MDSCs suppress the CD8<sup>+</sup> T cell response[36].  
S100A9 is a marker that can be used to distinguish MDSCs from monocytes[37]. Studies have identified CD14<sup>+</sup>HLA-DR<sup>low</sup>/neg monocytes as MDSCs in HCC patients. The frequency of CD14<sup>+</sup>HLA-DR<sup>low</sup>/neg MDSCs in the peripheral blood and ascites fluid of these patients is increased. CD14<sup>+</sup>HLA-DR<sup>low</sup>/neg MDSCs can not induce T cell proliferation. MDSCs induce FoxP3<sup>+</sup> regulatory T (Treg) cell proliferation, while CD14<sup>+</sup>HLA-DR<sup>+</sup> monocytes induce Th17 cell proliferation and IL-17 secretion[33]. MDSCs induce Treg cell generation via all-trans retinoic acid (ATRA)[38]. In human HCC, the fibrotic tissue around the tumor is rich in mMDSCs, which are significantly associated with a low survival rate[26].

#### Dendritic cells (DCs) and HCC

Dendritic cells (DCs) are a class of bone marrow-derived cells in the blood, epithelia and lymphoid tissues and are the most powerful professional antigen-presenting cells (APCs)[39]. DCs participate in the regulation of innate and adaptive immunity[40]. DCs have antigen delivery capabilities, which make them attractive carriers for therapeutic tumor vaccines and platforms for vaccine development[41]. Human DCs are usually induced from monocytes isolated from peripheral blood mononuclear cells (PBMCs) by M-CSF and IL-4 stimulation and develop into mature DCs (mDCs) after being loaded with antigens[42].

Human DCs express CD45 and HLA-DR and are divided into different populations according to CD11c, CD123 and IL-3R $\alpha$  expression. Myeloid DCs (mDCs) are important antigen-presenting cells and express high levels CD11c but low levels of CD123. Plasmacytoid DCs (pDCs) express high levels of CD11c, CD123, Toll-like receptor (TLR)-7 and TLR-9[43,44]. In normal liver tissues, CD141<sup>+</sup> hepatic DCs express CLEC9A, ILT3 and ILT4, but cell percentages and functions are damaged in hepatocellular carcinoma[45-47]. Evidence indicates that DCs have high antitumor and cytotoxic activity against HCC cells in vitro and in vivo[48]. DCs inhibit the growth of HCC cells

by loading LCSC antigens[49].

Peptide-pulsed DCs present antigens to naive T cells, thereby activating and inducing <sup>18</sup> naive T cells to become antigen-specific cytotoxic T lymphocytes (CTLs) to kill tumor cells[50]. DCs incubated with epithelial adhesion molecule (Ep-CAM) peptides have a significant inhibitory effect on tumor growth[51]. DC precursors that are sensitized by dexamethasone can effectively trigger the <sup>4</sup> major histocompatibility complex class I (MHC I)-restricted CTL response, allowing DCs to make full use of secondary antigen peptides, thereby maximizing the specificity of the HCC immune response[52].

Macrophages and HCC

Macrophages originating in the liver are generally described as Kupffer cells (KCs)[53]. <sup>21</sup> KCs express MHC class II and have <sup>2</sup> varying levels of costimulatory markers, such as CD40, CD80, CD86, and express the inhibitory marker Z39Ig[20,54]. The activation of STAT3 <sup>10</sup> is involved in the differentiation of monocytes into macrophages[55]. Although tumor-infiltrating macrophages are generally believed to be derived from circulating monocytes, emerging evidence suggests that tissue macrophages can be maintained through self-renewal[56]. Liver resident macrophages are established by progenitor cells derived from the fetal liver and are maintained by self-proliferation and monocyte migration[57,58]. KCs in the liver are negatively correlated with patient prognosis by inhibiting T cell antitumor functions[59].

<sup>13</sup> Tumor-associated macrophages (TAMs) are the most abundant immune cells in the tumor microenvironment and play a key role in immunosuppression[60]. TAMs are important in tumor-related inflammation and can be polarized into M1 or M2 phenotypes[61]. <sup>3</sup> M1 macrophages are induced by LPS and IFN- $\gamma$ ; M2 macrophages are induced by IL-4 and IL-13[62]. It is generally believed that M1 TAMs <sup>3</sup> have antitumor functions, while M2 TAMs promote tumor proliferation[63]. Studies have shown that IL-10 is involved in the polarization of TAMs to the M2 phenotype[64]. CD68

and CD163 are two popular TAM cell surface markers[65,66]. It has been reported that CD68+HLA-DR+ TAMs enhance HCC proliferation through the NFκB/FAK pathway[67].

TAMs induce CD8+ T-cell exhaustion<sup>26</sup> via the PD-1/PD-L1 and Tim3 signaling pathways in the tumor microenvironment[68,69]. CCR2+ TAMs accumulate and express the inflammatory marker S100A9 at the border of highly vascularized HCC, while CD163+ TAMs accumulate in the center area of HCC tissue[70]. Silencing SIRT4 in TAMs regulates macrophage activation and significantly promotes HCC cell growth[71]. Down-regulation of SIRT4 expression is related to an increase in macrophage infiltration in HCC tissues<sup>14</sup> and a high ratio of M2/M1 macrophages[72]. The miR-148B/CSF1 pathway<sup>12</sup> regulates the entry of TAMs into HCC tissue. A lack of miR-148B induces CSF1 expression and macrophage infiltration<sup>12</sup>, thereby promoting liver cancer metastasis and poor prognosis[73].

The regulatory function of HCC on the MPS

Studies have shown that the tumor environment can trigger early activation of monocytes in the surrounding tumor tissues[74,75]. Tumor environmental factors induce monocytes to express PD-L1 transiently in the early stage, activate monocytes, and induce PD-L1 expression in an autocrine cytokine-dependent manner[75]. HCC cells promote monocyte-to-macrophage differentiation via the PKM2 pathway[76]. In addition, monocytes in human peripheral blood differentiate into immature dendritic cells[77]. Monocyte-derived TNF-α<sup>6</sup> works synergistically with tumor-derived soluble factors to induce neutrophils to produce the metastasis-promoting factor OSM. Tumor-infiltrating monocytes mediate the production of CXCL2 and CXCL8 through the PFKFB3/NFκB signaling pathway<sup>6</sup>. The level of PFKFB3 and the production of CXCL2/CXCL8 in monocytes are positively correlated with the infiltration of OSM-producing neutrophils in human HCC tissues<sup>6</sup>[78].

The tumor microenvironment inhibits DC maturation and activation, leading

to an immunosuppressive phenotype and function[79]. In addition, hepatoma cells recruit immunosuppressive DCs and MDSCs to suppress CD8<sup>+</sup> T cells, leading to tumor escape from immune surveillance[80]. Tumor-derived exosomes impair the differentiation and maturation of DCs through the IL-6/STAT3 signaling pathway, which prevents myeloid precursor cells from differentiating into CD11c<sup>+</sup> DCs and induces apoptosis, thereby reducing T cell activation and mediating immunosuppression[81,82].

The proliferation of tumor infiltrated macrophages is more powerful than that of macrophages in nontumor tissues. Increased levels of macrophage proliferation are positively correlated with the density of macrophages in the tumor and the poor prognosis of HCC patients. Proliferating macrophages can be induced by small soluble adenosine, which is derived from tumor cells. GM-CSF released by tumors stimulates macrophages to express the A2A receptor, which then coordinates with adenosine to cause HCC-infiltrating macrophage proliferation[83]. Mitochondrial nucleoid structural changes lead to the release of mitochondrial DNA (mtDNA) into the cytoplasm, which is considered to be mitochondrial DNA stress, thereby regulating innate immunity[84].

CSF-1 and CCL2 in the tumor microenvironment polarize macrophages to the M2 phenotype[85,86]. Crosstalk between macrophages and liver tumor cells can also affect the polarization of TAMs through the Wnt/ $\beta$ -catenin signaling pathway[87]. Compared to those in M1 cells,  $\beta$ -catenin, c-Myc and Axin2 are more enriched in M2 TAMs. It is believed that the c-Myc signaling pathway promotes the polarization of TAMs toward the M2 phenotype[88]. Neurotensin (NTS)-induced IL-8 polarizes TAMs to the M2 phenotype and promotes epithelial-mesenchymal transition (EMT) in HCC through the MAPK and NF $\kappa$ B pathways[89]. In addition, hypoxia-inducible factors 1 $\alpha$  (HIF-1 $\alpha$ ) and HIF-2 $\alpha$  cause TAM polarization through hypoxia in the tumor microenvironment, leading to HCC malignancy[90,91]. Hypoxia-induced high mobility group protein box 1 (HMGB1) may affect tumor progression by

regulating TAMs, leading to poor clinical prognosis[92].

## CONCLUSION

Components of the MPS have important but distinct roles in HCC. The MPS is associated with specific cell phenotypes and functions in the HCC microenvironment. Monocytes are progenitors of DCs and macrophages, which play an important role in inhibiting the immune response. In addition, MDSCs are a heterogeneous population composed of immature myeloid cells and myeloid progenitor cells. Increasing evidence suggests that tumor progression is associated with the accumulation of MDSCs, which cause local and systemic immunosuppression[93]. Furthermore, increased accumulation of MDSCs is associated with early tumor recurrence and is considered to be one of the predictors of poor prognosis in HCC patients who undergo clinical treatment[94]. Cytokine-induced killer (CIK) cells include a wide variety of TCR-specific effector cells, which are a mixed cell population. CIK cells have cytotoxic activity against tumor cells, which is not MHC-restricted. Studies have shown that adjuvant immunotherapy for HCC using CIK cells can reduce tumor recurrence and improve overall survival[95]. MDSCs inhibit CIK cell lysis via ARG1 and iNOS.

Human DCs are usually induced from monocytes isolated from peripheral blood. DCs have antigen delivery abilities, which make them attractive carriers for therapeutic tumor vaccines and platforms for vaccine development. In normal liver tissues, hepatic DCs have powerful antigen presentation functions and immunomodulatory abilities, but their percentages and functions are damaged in hepatocellular carcinoma[45]. Monocytes act as precursor cells that differentiate into DCs and macrophages under normal physiological conditions. Will monocyte impairments be inherited by DCs and macrophages? Will DCs and macrophages acquire immunosuppressive phenotypes and functions in HCC after differentiation? These questions remain to be investigated.

Although most previous studies have focused on each MPS component,

interactions and cooperation among various MPS components are common[96-98]. For instance, KCs are the main immune cells in liver tissues and play a key role in DC recruitment to the liver[97]. pDC survival and IFN- $\alpha$  production are affected by IL-10 and TNF- $\alpha$  secretion by monocytes. MDSCs produce increased levels of IL-10, which leads to pDC apoptosis. How do MDSCs interact with pDCs in the HCC microenvironment? Additionally, monocyte- or macrophage-derived IL-15 and pDC-derived IFN- $\alpha$  synergistically stimulate IFN- $\gamma$  production in NK cells during HCV infection[96]. Does this interaction occur in HCC? Other forms of cooperation between different MPS components remain to be analyzed.

In this review, the impact of HCC on each component of the MPS and the immune function of the MPS in HCC development were described. Monocytes are precursors of DCs and macrophages, which play an important role in the regulation of immune function in HCC. Through crosstalk with hepatoma cells or effects of the tumor microenvironment, monocytes are capable of producing high levels of IL-10, resulting in an inhibitory microenvironment. DCs can suppress the stimulation of T cells and inhibit efficient antitumor T-cell functions. TAMs are the most abundant immune cells in the tumor microenvironment and play a key role in immunosuppression. The tumor microenvironment inhibits DC maturation and activation, leading to an immunosuppressive phenotype and function. The HCC microenvironment remodels MPS cell phenotypes and cytokine production and impairs MPS components in HCC.

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24 &NA;. "Abstracts for the 25th Annual Scientific Meeting of the International Society for Biological Therapy of Cancer :", Journal of Immunotherapy, 10/2010  
Crossref

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25 Pablo A. Vieyra-Garcia, Peter Wolf. "A deep dive into UV-based phototherapy: Mechanisms of action and emerging molecular targets in inflammation and cancer", Pharmacology & Therapeutics, 2021  
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26 d-scholarship.pitt.edu  
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27 www.freepatentsonline.com  
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28 Zhe (Jenny) Zhang, Peggy A Bulur, Ahmet Dogan, Dennis A Gastineau, Allan B Dietz, Yi Lin. " Immune independent crosstalk between lymphoma and myeloid suppressor CD14 HLA-DR monocytes mediates chemotherapy resistance ", Oncolmunology, 2015  
Crossref

---

29 Firouzeh Korangy, Bastian Höchst, Michael P Manns, Tim F Greten. "Immunotherapy of hepatocellular carcinoma", Expert Review of Gastroenterology & Hepatology, 2014  
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