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Review Article

Tumor-Associated Macrophages in Hepatocellular Carcinoma: Friend or Foe?

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Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, and it has diverse etiologies with multiple mechanisms. The diagnosis of HCC typically occurs at advanced stages when there are limited therapeutic options. Hepatocarcinogenesis is considered a multistep process, and hepatic macrophages play a critical role in the inflammatory process leading to HCC. Emerging evidence has shown that tumor-associated macrophages (TAMs) are crucial components defining the HCC immune microenvironment and represent an appealing option for disrupting the formation and development of HCC. In this review, we summarize the current knowledge of the polarization and function of TAMs in the pathogenesis of HCC, as well as the mechanisms underlying TAM-related anti-HCC therapies. Eventually, novel insights into these important aspects of TAMs and their roles in the HCC microenvironment might lead to promising TAM-focused therapeutic strategies for HCC. **(Gut Liver 2021;15:500-516)**

Key Words: Hepatocellular carcinoma; Tumor-associated macrophages; Macrophage polarization; Epigenetic modification; Cancer therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, and it is a disease with a heavy global public health burden because of its increasing incidence and high mortality.[1] HCC is a multistep and heterogeneous process characterized by rapid progression and poor prognosis, which is at least partially explained by high resistance and recurrence.[2] Although there is a wide range of available therapeutic options for HCC, many radical therapies are not satisfactory, including surgical resection, transplantation, radiotherapy, local radiofrequency ablation, chemotherapy and interventional therapies (transarterial chemoembolization). These aforementioned remedies have limited effectiveness due to the accumulation of molecular and cellular alterations in HCC.[3] In view of the disadvantages of the conventional strategies, therapies that utilize immunotherapies alone or in combination with molecularly targeted therapies are currently considered required tools for precision medicine-based treatment of HCC,[4] particularly for advanced-stage HCC. Recent clinical trials have demonstrated that HCC immunotherapies, including pembrolizumab, atezolizumab and bevacizumab, are emerging as tools to boost the antitumor immune response and promote overall and progression-free survival outcomes in patients with unresectable HCC.[5-7] Notably, T cell-based HCC immunotherapy with immune checkpoint inhibitors (ICIs) has been proven to be efficient, such as the cytotoxic lymphocyte antigen 4 (CTLA-4) or programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway.[8] However, only a small portion of patients benefit from these anti-HCC therapies, which are accompanied by resistance and several immune-related adverse events,[9] highlighting that novel approaches and targets are urgently needed to produce clinically effective and safe treatments of HCC.

Mirroring the Th1/Th2 nomenclature and paradigm, the continuum of polarized macrophages is commonly referred to as classically activated macrophages (M1) or alternatively activated macrophages (M2), representing two ex-

tremes of a dynamic changing state that occur in response to diverse microenvironmental signals.[10] According to this dichotomous model, M1-type macrophages are stimulated by the Th1-related cytokine interferon-γ (IFN-γ) alone or in combination with microbial stimuli lipopolysaccharide (LPS) or cytokines tumor necrosis factor-α (TNF-α) and granulocyte-macrophage colony stimulating factor (GM-CSF); these factors are generally supportive of pro-inflammatory/anti-tumorigenic responses.[11] However, M2-type macrophages are induced by Th2-derived mediators interleukin (IL)-4 or IL-13 and M-CSF (M2a), immune complexes and agonists of Toll-like receptors (TLRs) or IL-1R (M2b), IL-10 and glucocorticoid hormones (M2c), and they are involved in promoting anti-inflammatory/pro-tumorigenic responses.[12,13] Hepatic macrophages consist of self-renewing tissue-resident macrophages in the liver, termed Kupffer cells (KCs), which originate from the fetal yolk sac, as well as infiltrated hematopoietic stem cells/bone marrow-derived monocytes. They are still a remarkably heterogeneous population (M1/M2 KCs or infiltrated macrophages) and play key roles in liver homeostasis and diseases, effecting processes such as inflammation, organ injury, fibrosis, and other pathological processes, which have been covered in other systematic reviews.[14,15] Targeting these different liver macrophage subpopulations and their phenotypic switch with pharmacologic or genetic approaches, exhibiting potential therapeutic effects, may help in the development of new alternative therapies to better treat HCC.

The field of cancer immunotherapy, including therapies for HCC,[16] is moving fast because of encouraging clinical results hallmarked by prolonged survival compared with that of traditional remedies.[17] However, current immunotherapeutic approaches are still limited, and a great need still exists for identifying novel targets to treat HCC.[18] More recently, tumor-associated macrophages (TAMs) being found at a high density has been frequently associated with poorer prognosis and serves as a potential diagnostic and prognostic biomarker in many cancers.[19] TAMs are major components of the innate immune system and will likely be useful in HCC immunological therapies because they modulate the tumor microenvironment (TME).[20] Recent immune-genomic analysis utilizing data compiled by TCGA has classified HCC as a C4 subtype, which is characterized by enrichment of M2 macrophages and suppression of the Th1 $CD4^+$ T cell response.[21,22] For example, a *Listeria*-based HCC vaccine combined with anti-PD-1 immunotherapy exerted synergistic antitumor effects by skewing TAM polarization from M2 into M1 and by facilitating T-cell reactivity.[20] Consequently, targeting TAM function and polarization in the TME of HCC might be developed as preventive and therapeutic strategies against this deadly disease. In HCC, very recent data using multiomics approaches demonstrated significant heterogeneity in the immune microenvironment.[23,24] Here, we review multiple signaling pathways, functional signatures and molecular mechanisms implicated in shaping TAM activation and function as they relate to the pathophysiologic processes of HCC, together with the advancements achieved by the management of TAMs and orchestrating functions of other cell types in HCC clinical therapies.

FUNCTIONAL CHARACTERIZATION OF M2 TAMs IN HCC

TAMs are well established as key components of the complex TME ecology and are influenced by tumor-derived cytokines to promote malignancy and progression in various tumors.[25] TAMs have been suggested to exhibit significant immunosuppressive effects and to generally play a pro-tumoral role by acting as a driver of M2 polarized macrophages, leading to tumor growth, immunosuppression, angiogenesis, invasion, and metastasis.[26]

KCs and infiltrated monocytes are the main source of mononuclear phagocytes in the liver microenvironment. Upon liver inflammation, injury, and infection, they can become rapidly polarized into specific phenotypes adapted to the local microenvironmental factors.[27] If unresolved, they can progress to cause fibrosis, cirrhosis and/or HCC, by which macrophage immunomodulation is an indispensable tool for understanding and evaluating the pathophysiology of liver diseases.[28] Different etiologies (LPS, CCl_4, hepatitis viruses, alcohol, fat and other inducers) cause persistent liver injury, and fibrosis might be strongly associated with the initiation of HCC.[29] Suppression of the pro-inflammatory response by KCs could inhibit the initiation of HCC but could promote the progression of HCC.[30] As determined by Lee *et al.*,[30] in accordance with enhanced inflammation, there were significant increases in hepatic inflammation, fibrosis and tumor growth in mice fed a high-fat diet, which might be attributed to the pro-inflammatory, pro-fibrogenic and pro-tumoral responses mediated by vascular endothelial growth factor-dependent angiogenesis of KCs.[31] It is therefore inferred that liver macrophage phenotypes are not fixed and that their dynamic alterations are implicated in hepatocarcinogenesis and its progression. As expected, similar to the TAM phenotype features observed in other cancers, the TAM subpopulation in HCC is predominantly of the M2 subtype, which is an important promoter for tumor initiation and progression. For example, Nogo-B was suggested to play

a role in facilitating TAM M2 polarization and promoting the pro-tumoral effects of TAMs via the Nogo-B/yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) pathway in HCC.[32] Consistently, interrupting YAP function by statins could improve HCC treatment by suppressing IL-6-mediated TAM recruitment.[33] The downregulation of the tumor suppressor gene SIRT4 in TAMs skewed M2 activation and promoted HCC development via the fatty acid oxidation-peroxisome proliferator-activated receptor (PPAR)δ-signal transducer and activator of transcription 3 (STAT3) axis.[34] Either pharmacologic depletion of this population[35] or reprogramming the polarity of TAMs from M2 toward the M1 phenotype resulted in the potential to suppress HCC progression.[36] For example, oxidored-nitro domain-containing protein 1-deficient mice displayed resistance to diethylnitrosamine (DEN)-induced HCC, which was dependent on suppression of M2 in favor of M1 TAM polarization and KC infiltration.[37] There is no doubt that uncovering the molecular mechanisms underlying the TAM phenotypic switch and the spatial-temporal variation of infiltration is a promising strategy for treating HCC (Fig. 1).

EPIGENETIC MODIFICATION OF TAMs AND TUMOR CELLS IN HCC

1. Noncoding RNAs

Noncoding RNAs (ncRNAs) are non-protein-coding RNAs, and they are emerging as major regulators of a great variety of biological processes, including gene expression, cell proliferation and differentiation.[38] MicroRNAs (miRNAs), long ncRNAs (lncRNAs) and circular RNAs account for the vast majority of ncRNA regulatory networks, and ncRNA-related TAM function and polarization are required for tumorigenesis in many solid[39] and nonsolid[40] tumors. As expected, recent genomic and transcriptomic projects have unraveled the presence of a large number of ncRNAs linked to hepatic carcinogenesis in humans and mice, and they function in part through modulating TAM

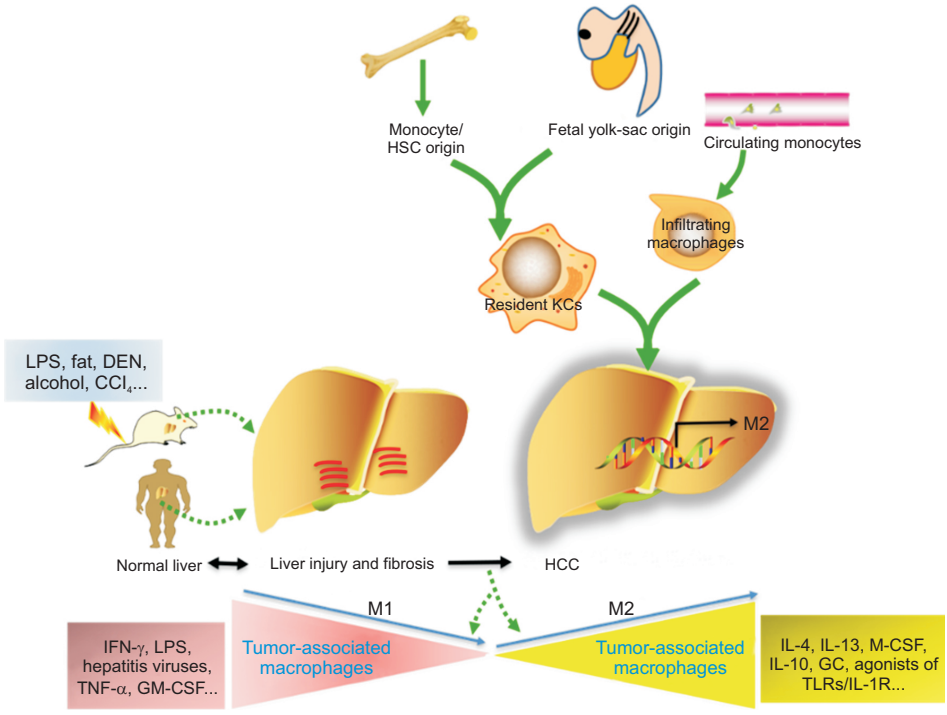

Fig. 1. A schematic diagram depicting the disparate origins and phenotypes of heterogeneous macrophages in hepatocellular carcinoma (HCC). HCC is one of the deadliest cancers worldwide, and liver fibrosis is a key factor in the development of HCC. Chronic liver injury due to different etiologies, including lipopolysaccharide (LPS), CCl4, a diet rich in fat/alcohol, diethylnitrosamine (DEN) and others, leads to liver fibrosis that can ultimately develop into HCC. Hepatic macrophages (infiltrated macrophages and resident Kupffer cells [KCs]) originate from fetal yolk sac and infiltrated hematopoietic stem cells (HSCs)/bone marrow-derived monocytes. Emerging evidence suggests the pivotal role of heterogeneous macrophages in the development of liver injury, fibrosis and HCC. The predominant M1 macrophages in liver fibrosis could be reprogrammed into M2-activated phenotypes in response to stimuli from the tumor microenvironment. We propose that tumor-associated macrophages may represent a target for the prevention or treatment of HCC.
IFN-γ, interferon-γ; IL, interleukin; GC, glucocorticoid hormones; GM-CSF, granulocyte-macrophage colony stimulating factor; M-CSF, macrophage colony-stimulating factor; TLR, Toll-like receptor; TNF, tumor necrosis factor.

functional plasticity.[41]

miRNAs are one of the most evolutionarily conserved types of ncRNAs, and emerging evidence supports the pivotal roles of miRNA in macrophage polarization during HCC pathogenesis. The symbiotic relationship and crosstalk between TAMs and tumor cells in direct and/or indirect ways have been thoroughly revealed in HCC. Human HCC HepG2 cells promoted both human leukemia monocytic cell line (THP-1) recruitment and differentiation into macrophages, promoting matrix metalloproteinases (MMP)-2 and -9 expression through THP-1, which increased proliferation of HepG2 cells.[42] MMP-9 activity is strongly related to the growth of many cancers,[43] and M2 macrophage-mediated miR-149-5p inhibition and accelerated MMP-9 expression in HCC cells promote HCC progression.[44] Higher expression of miR-155 was observed in the tumor region linked with pathogenesis and therapy in various cancer types.[45] Consistently, aberrantly high expression of miR-155 was detected in both human HCC tissues and cell lines, which promoted tumor growth by targeting the AT-rich interactive domain 2 (ARID2)-mediated Akt phosphorylation pathway.[46] Nevertheless, whether the dysregulation of miR-155 exists in liver TAMs and is predictive of a pro- or anti-tumorigenic response is unclear. Upregulation of the lncRNA cox-2 skewed the macrophage phenotype from M1 to M2, and M2 TAMs facilitated HCC cell growth by promoting HCC immune evasion.[47] These observations demonstrated that ncRNA-mediated TAM polarization is implicated in HCC.

Exosomes secreted by host cells play an important role in intercellular communication of the HCC TME, and exosome-derived ncRNAs are associated with hepatocarcinogenesis.[48] HCC cell-secreted exosomes were found to contain elevated levels of lncRNA TUC339 and to contribute to M2 TAM polarization by enhancing multiple signaling pathways.[49] However, the underlying mechanisms by which TUC339 promotes HCC progression have not been fully addressed. The Sal-like protein-4 (SALL4)/miR-146a-5p axis in HCC exosomes was shown to promote M2 polarization and T cell exhaustion, resulting in HCC progression.[48] In turn, high miR-125a/b-expressing TAM-derived exosomes suppressed HCC cell proliferation and stem cell properties through targeting of CD90 by miR-125a/b to reduce its expression.[50] The significance of tumor-suppressive miR-125b in HCC was further revealed when it was shown to be involved in decreasing histone methylation and tumorigenicity.[51] Several lines of evidence indicate that deregulation of miRs is closely related to TAM infiltration and poor clinical outcomes in HCC. Upregulation of IL-34 due to decreases in miR-28-5p in HCCs led to TAM infiltration, which further inhibited miR-28-5p expression in HCC cells through the activity of transforming growth factor beta 1; this resulted in a positive feedback loop, resulting in a poor prognoses for patients.[52] Ke *et al.*[53] found that miR-148b deletion promoted HCC growth and metastasis through colony stimulating factor-1 (CSF1)/CSF1 receptor (CSF1R)-mediated TAM infiltration. Most importantly, the biological characteristics of HCC cells were not affected by miR-28-5p or miR-148b deficiency *in vitro*, suggesting that the biofunctional roles of miRs in HCC depend on TAM infiltration.[52,53] Consistently, Hu *et al.*[54] identified circASAP1 (a circRNA derived from exons 2 and 3 of the ASAP1 gene, hsa_circ_0085616) as a key promotor of HCC metastasis that enhanced TAM infiltration, and they also showed that the process was dependent on miR-326/miR-532-5p-mitogen-activated protein kinases 1 (MAPK-1)/CSF-1 signaling. These results supported the critical role of ncRNA-regulated CSF in TAM infiltration of HCC, which deepened our understanding of how ncRNA functions in HCC progression. $CD68^+$ TAM-induced overexpression of lncRNA H19 resulted in poor prognoses because it promoted HCC cell invasion through activating the miR-193b/MAPK1 axis.[55] Eventually, ncRNA-directed TAM infiltration and polarization may be developed as novel potential ncRNA-based therapies for HCC (Table 1).

2. DNA methylation

DNA methylation regulation of gene promoters or enhancers has been recently implicated in disrupted gene expression in liver diseases, including HCC.[56,57] There is now increasing evidence that altered DNA methylation plays a pivotal role in TME remodulation of HCC by influencing macrophage infiltration and differentiation.

DNA hypomethylation might contribute to the overexpression of centrosomal protein 55 (CEP55), which was closely correlated with the infiltration level of macrophages, predicting poorer clinical outcomes in patients with liver cancer.[58] Deregulation of angiopoietin-like 4 (ANGPTL4) in HCC is caused by a high concentration of methylated of CpG sites in the ANGPTL4 promoter, which was significantly associated with advanced tumor stage.[59] Treatment with Ad-ANGPTL4 significantly inhibited the development of HCC, which occurred partially by destroying the tumor-favorable microenvironment, including decreased $CD68^+$ macrophage infiltration and alterations in the profile of cytokines secreted from macrophages in the TME.[59] Macrophage-related chemokines have been proposed as novel molecular targets for HCC, such as C-X-C motif chemokine ligand 2 (CXCL2). However, the expression profile of CXCL2 remains controversial. CXCL2 was confirmed to have higher expression in a coculture system with M2 and SMMC7721 cells as well as HCC tis-

Table 1. Epigenetic Modification-Mediated TAM Function and Polarization Linked to the Pathogenesis of HCC

Author	Name	Expression	Effects on TAMs	Effects on HCC	Potential targets/mechanisms
Liu *et al.*[44]	miR-149-5p	↓	M2 polarization↑	↑	MMP9
Zhang *et al.*[46]	miR-155	↑	?	↑	ARID2
Ye *et al.*[47]	Cox-2	↑	M2 polarization↑	↑	?
Yin *et al.*[48]	miR-146a-5p		M2 polarization↑	↑	SALL4
Li *et al.*[49]	TUC339	↑	M2 polarization↑	?	TLR signaling
Wang *et al.*[50]	miR-125a/b	↓	Exosome→	↑	CD90
Fan *et al.*[51]			?	↑	SUV39H1
Zhou *et al.*[52]	miR-28-5p	↓	Infiltration↑	↑	IL-34
Ke *et al.*[53]	miR-148b	↓	Infiltration↑	↑	CSF1
Hu *et al.*[54]	circASAP1	↑	Infiltration↑	↑	miR-326, -532-5p
Ye *et al.*[55]	H19	↑	Infiltration↑	↑	miR-193b/MAPK1
Yang *et al.*[58]	CEP55	↑	Infiltration↑	↑	DNA hypomethylation
Ng *et al.*[59]	ANGPTL4	↓	Infiltration↑	↑	Hypermethylation of CpG sites of promoter
Lu *et al.*[60]	CXCL2	↑	M2 polarization↑	↑	?
Ding *et al.*,[61] Subat *et al.*[62]		↓	?	↓	DNA hypermethylation
Tikhanovich *et al.*,[66,67] Zhao *et al.*[68]	PRMT1	↑	M2 polarization↑	↑	histone H4R3me2a methylation of PPARγ
Wei *et al.*,[70] Yin *et al.*[71]	CXCL10/CXCR3	↑	M2 polarization↑	↑	DNMT1, EZH2

TAM, tumor-associated macrophage; HCC, hepatocellular carcinoma; M2, macrophage; MMP9, matrix metalloproteinases 9; ARID2, AT-rich interactive domain 2; Cox-2, cyclooxygenase 2; SALL4, Sal-like protein-4; TLR, Toll-like receptor; SUV39H1, suppressor of variegation 3-9 homolog 1; IL-34, interleukin-34; CSF1, colony stimulating factor-1; circASAP1, a circRNA derived from exons 2 and 3 of the ASAP1 gene, hsa_circ_0085616; MAPK1, mitogen-activated protein kinase 1; CEP55, centrosomal protein 55; ANGPTL4, angiopoietin-like protein 4; CXCL2, C-X-C motif chemokine ligand 2; PRMT1, protein arginine methyltransferase 1; PPAR, peroxisome proliferator-activated receptor; CXCR, CXC chemokine receptor; DNMT1, DNA methyltransferase 1; EZH2, histone H3 lysine 27 methyltransferase; ↑, promoting effect; ↓, inhibitory effect; ?, unknown effect or unknown targets.

sues, and it was found to promote the metastasis of HCC.[60] Conversely, to determine whether the decreased CXCL2 in HCC[61,62] was controlled by DNA methylation, after treating HCC cell lines were treated with the DNA demethylating agent 5-aza-2'-deoxycytidine, and upregulated CXCL2 levels were observed.[62] These findings might indicate that in HCC, the down- or upregulation of CXCL2 by different TAMs or cancer cells is associated with aberrant DNA methylation; however, further studies are warranted to determine accurate expression patterns. Altogether, the identification of DNA methylation-associated TAM activation could provide further insights into the pathogenesis of HCC (Table 1).

3. Histone modification

An altered pattern of histone modifications (methylation, phosphorylation, acetylation, glycosylation and other modifications) is central to various liver diseases, including HCC.[63] Most importantly, there is increased attention on histone modifications that impact hepatic macrophage functional responses and M1/M2 polarization by modulating cellular signaling and signature gene expression.[64,65]

Protein arginine methyltransferase 1 (PRMT1) is known to be an important regulator of inflammatory responses[66] and is required for favoring an anti-inflammatory M2 phenotype through histone H4R3me2a methylation of the PPARγ promoter.[67] Moreover, PRMT1-dependent arginine methylation is necessary for c-Myc function in M2 differentiation, resulting from c-Myc binding to the acetyltransferase p300 and from a decrease in histone deacetylase 1 (HDAC1) recruitment.[66] PRMT1 expression in TAMs correlates with STAT3 activation in human and mouse HCC specimens, and the activation of the PRMT1-IL-6-STAT3 axis is an important mechanism in alcohol-associated tumor progression.[68] These data suggested that PRMT1-dependent M2 polarization was attributed to dysregulation of histone modifications and may be useful in testing the pathologic mechanisms of HCC. The liver inflammation context at the tumor site can markedly influence the biological behavior of a malignant tumor, which is an important tumorigenic process.[69] Wei *et al.*[70] recently demonstrated that activated $CD4^+$ T cells stimulated pro-tumorigenic macrophage activation and promoted IgG^+ plasma cell polarization in a CXCL10/CXC chemokine receptor 3 axis-dependent manner. This process correlated with increased expression of DNA methyltransferase 1 (DNMT1) and histone H3 lysine

27 methyltransferase (EZH2);[70] however, they did not address whether and how DNMT1/EZH2 played roles in TAM polarization. Previous studies showed that DNMT1 and EZH2 might induce M1[70] or M2[71] polarization, respectively. In summary, although it is less clear how macrophages are reprogrammed during polarization to alter their responses to TME challenges, a deeper understanding of histone regulation of the macrophage phenotypic transition will enable the development of novel therapeutic approaches to HCC (Table 1).

GUT MICROBIOTA MODULATES TAM FUNCTION AND POLARIZATION IN HCC

Accumulating evidence suggests that the gut microbiota-liver axis influences hepatic innate immunity, potentially maintaining liver homeostasis and playing a role in pathologies.[72] The important link between gut microbiota and hepatocarcinogenesis can be observed by the gut microbiota profile (dysregulation of Enterobacteriaceae, Streptococcus, Akkermansia, etc.) and through systemic inflammation (upregulation of IL-8, IL-13, CCL3, CCL4, and CCL5) in patients with cirrhosis and nonalcoholic fatty liver disease that developed HCC because of these factors.[73]

TLRs function as crucial pattern recognition receptors and play a critical role in recognizing invading pathogens and initiating innate immune responses via the recognition of pathogen-associated molecular patterns.[74] Macrophages show great effects in eliminating microbes and initiating inflammatory responses through the TLR pathway.[75] Notably, the macrophage-expressed serine/threonine-protein kinase 4 (STK4)-mediated anti-inflammatory response could prevent LPS or *Escherichia coli* infection-associated HCC; likewise, macrophage-specific STK4 deficiency resulted in chronic inflammation, liver fibrosis, and HCC in mice treated with a combination of DEN and CCl_4 and exposed to pathogenic infection.[76] STK4 could act as an HCC suppressor by selectively inhibiting TLR4/9-induced pro-inflammatory cytokine secretion and enhancing TLR3/4-triggered IFN-β production; the function depends on the phosphorylation and degradation of IL-1 receptor-associated kinase 1.[76] Currently, it is thought that gut microbiota-mediated TLR signaling is largely considered to induce inflammatory and fibrogenic responses, contributing to HCC tumorigenesis.[77,78] Moreover, TLR4 (but not TLR2) on macrophages was required for the tumor growth of steatohepatitis-related HCC in mice, which could be inhibited by gut sterilization via treatment with an antibiotic mixture.[78,79]

Apart from the potential etiology of HCC, the gut microbiome may solve the difficulties of factors affecting and predicting the response to immunotherapy in HCC. For example, Zheng *et al.*[80] demonstrated that patients responding to anti-PD-1 immunotherapy contained different *Proteobacteria*, *Akkermansia muciniphila* and *Ruminococcaceae* spp., compared with those of nonresponders. Unfortunately, the potential functions of macrophages and TLRs were not discussed in this study. Targeting gut microbiota with antibiotics and probiotics could be a promising therapeutic approach for treating HCC because they would alter intestinal immune cell migration and function.[81] Patients on a diet that included probiotics exhibited reduced intestinal IL-17 production and exhibited greater promotion of differentiation of anti-inflammatory regulatory T cell (Treg)/Tr1 cells, which potentially suppressed HCC progression.[82] In conclusion, understanding of the diagnostic and therapeutic potential of macrophages in HCC by targeting the crosstalk between the gut and liver is urgently needed.

TAMs IN HCC THERAPY

1. Chemotherapy and molecule-targeting therapy

Monotherapy and multidrug treatment with drugs such as platinum-based drugs (cisplatin and oxaliplatin), doxorubicin,[83] and gemcitabine,[84] are important chemotherapeutic and chemoimmunotherapeutic options for HCC. Further, as recommended by international guidelines, updated Barcelona Clinical Liver Cancer treatment algorithms and multikinase inhibitors (sorafenib and lenvatinib) are now feasible as a first-line treatment for advanced HCC.[85] In addition, regorafenib, cabozantinib and ramucirumab are appropriate supplements as second-line treatments for patients with advanced HCC who are in poor condition.[86,87] However, tumor resistance to these chemotherapeutic, recurrence and disruption of relevant molecules are common clinicopathologic characteristics of HCC and have been major obstacles to improving the prognosis of patients with HCC. Here, we discuss recent developments in anti-HCC chemotherapy and chemoimmunotherapy based on macrophage function and polarization.

A variety of HCC drug-resistant processes and mechanisms have been identified by clinical treatment and laboratory-based studies, and they remain a major problem in the management of HCC. Of note, epithelial to mesenchymal transition (EMT),[88] autophagy,[89] and hypoxia[90] are now emerging as crucial players in the response to anti-HCC therapeutics, which can underlie clinical drug resistance.

Collective evidence shows that TAMs play essential roles in HCC therapy resistance by cross-talking with tumor cells involved in autophagy.[91] Fu *et al.*[92] suggested that TAMs inhibited oxaliplatin cytotoxicity in SMMC-7721 and Huh-7 cell lines and HCC xenografts in mice by inducing autophagy in HCC cells to avoid apoptosis, which might contribute to oxaliplatin resistance. However, TAM phenotypes in this process are poorly defined. Interestingly, an increase in the release of IL-17 from M2-TAMs was observed after oxaliplatin treatment for HCC, which stimulated chaperone-mediated autophagy and induced tolerance to oxaliplatin by reducing cyclin D1 expression.[93] Therefore, M2-TAMs might be involved in autophagy-associated chemorefractory liver cancer. High mobility group box 1 (HMGB1) plays a role in autophagy regulation in cancer cells, and it promotes marked resistance to cisplatin by protecting HCC cells from apoptosis through a positive HMGB1/nuclear factor kappa B (NF-κB)/hypoxia-inducible factor 1α (HIF-1α) feedback loop.[94] Studies have reported that HCC cell-derived HMGB1 facilitates peritumoral macrophage infiltration via HIF-1α; in turn, TAM-secreted IL-6 further promotes EMT in HCC cells, which exacerbates invasion and metastasis of HCC.[95,96] Furthermore, IL-1β that is released by M2-TAMs could result in HCC cell EMT and metastasis in a hypoxic microenvironment through the HIF-1α/IL-1β/TLR4 axis.[97] The IL-6/IL-6R signaling axis has been proven to enhance EMT and TAM M2 polarization in triple-negative breast cancer.[98] Coincidentally, activation of IL-6/STAT3 signaling promoted M2-type macrophage polarization, and the inhibition of IL-6/STAT3 mediated by anti-IL-6 was found to reduce tumor formation in HCC by reducing the number of M2 TAMs.[99] Macrophage-derived IL-8 may induce EMT in HCC cells via the IL-8-activated JAK2/STAT3/Snail pathway.[100,101] Tumor hypoxia-induced HMGB1 promoted M2-like TAM accumulation and an IL-10-rich milieu within melanomas.[102] TAMs promote cancer stem cell-like properties via TGF-β1-induced EMT,[103] which attenuates Neferine-mediated oxaliplatin sensitization in HCC.[104] These results might indirectly suggest that HMGB1/HIF-1α and IL-6 signaling participates in autophagy and EMT by orchestrating the response of M2 TAMs and HCC cells, but this warrants further investigation of advanced HCC.

More recently, M2 (but not M1) TAMs have been observed to confer significant tumor resistance to sorafenib by secreting hepatocyte growth factor (HGF) and activating HGF/c-Met, MAPK/ERK1/2, and PI3K/AKT pathways in tumor cells, which in turn further enhanced M2 TAM infiltration and produced a positive feedback loop.[105] The CCL2/CC motif chemokine receptor–2 (CCR2) axis is required for the recruitment of monocytes/macrophages and M2 polarization of TAMs in HCC.[106] Herein, inhibition of the CCL2/CCR2 axis by treatment with a specific CCR2 antagonist played a role in preventing HCC.[106] This was supported by the robust attenuation of tumor-infiltrating macrophages (TIMs) and M2 TAM-mediated immune suppression as well as by the potentiated therapeutic effect of sorafenib, which was achieved by activating the $CD8^+$ T cell antitumoral response without inducing obvious toxicity.[107] Interestingly, patients with HCC had lower serum CCL2 levels than cirrhotic patients without HCC.[108] Twin-like core-shell nanoparticles were developed for the administration of a combined nanodrug delivery systems that included sorafenib and TAM repolarization agents,[109] which had great potential to be used in tumor-localized chemoimmunotherapy in clinics. It is obvious that ablation of the TAM population or skewing of the TAM phenotype from M2 to M1 could induce a drug-based anti-HCC response. Indeed, the antitumor activity of lenvatinib is derived from its immunomodulatory activity that is achieved by decreasing the proportion of monocyte and macrophage populations and increasing that of $CD8^+$ T cell populations.[110] Compound Kushen injection (CKI)-primed macrophages plus low-dose sorafenib treatment significantly promoted the proliferation and cytotoxic ability of $CD8^+$ T cells through TNFR1-mediated NF-κB and p38 MAPK signaling cascades, which subsequently resulted in apoptosis of HCC cells.[111] This study showed that CKI can potentiate chemotherapeutic drugs by inhibiting TAM-mediated immunosuppression,[111] which is a promising clinical chemo-immunotherapy strategy for liver cancer treatment. Eventually, TAMs coexist and interact with various immune cells (T cells, nature killer cells, neutrophils, etc.) to sustain the growth of HCC. The number of $CCL2^+$ or $CCL17^+$ tumor-associated neutrophils correlated with tumor growth, progression, and resistance to sorafenib, which occurred via the recruitment of macrophages and Treg cells to HCCs (Fig. 2).[112]

Taken together, understanding the role and mechanism of TAM biological processes is necessary to address the current problems related to drug-centered therapies for HCC.

2. Radiotherapy

Due to advancements in imaging technology, the beneficial roles of radiotherapy (RT) in precisely damping HCC development have been frequently revealed.[113] However, the efficacy and safety of RT alone have been limited by the relatively low liver tolerance to RT and dysregulation of TME following RT. Irradiation in HCC largely prevented tumor growth and caused continuous $F4/80^+CD68^+$ (M1) macrophage recruitment into irradiated tumors,[114,115] and

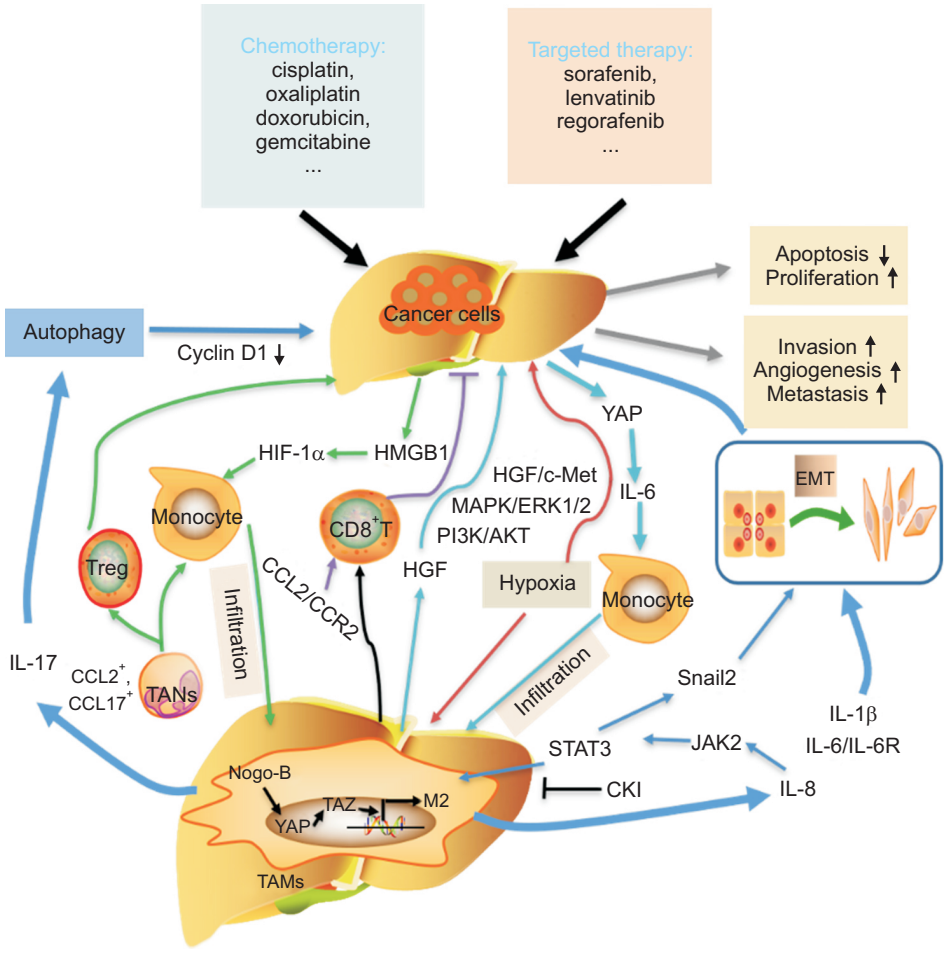

Fig. 2. Tumor-associated macrophages (TAMs) correlate with resistance to chemotherapy and molecular-targeted therapy in hepatocellular carcinoma (HCC). Chemotherapy and molecular-targeted therapy play critical roles in the treatment of HCC; however, tumor resistance frequently emerges during these therapeutic processes. Considerable evidence has shown that M2 TAMs are closely associated with therapeutic resistance. Targeting TAM infiltration and polarization in the HCC tumor microenvironment might result in significant antitumor activity in chemotherapy and molecular-targeted therapy for HCC.

AKT, protein kinase B (PKB); CCL, chemokine ligand; CCR2, CC motif chemokine receptor–2; CKI, compound Kushen injection; c-Met, hepatocyte growth factor receptor; ERK1/2, extracellular signal-regulated kinase 1/2; EMT, epithelial to mesenchymal transition; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; HMGB1, high mobility group box 1; IL, interleukin; JAK2, Janus kinase 2; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; Snail2, a zinc finger transcription factor 2; STAT3, signal transducer and activator of transcription 3; TANs, tumor-associated neutrophils; TAZ, transcriptional coactivator with PDZ-binding motif; Treg, regulatory T cell; YAP, yes-associated protein.

they induced an inflammatory response by producing TNF-α and IL-6.[115] In addition to altering the HCC immune microenvironment, infiltrated macrophages contributed to elevated glucose uptake after irradiation.[115] The increased TIM density was closely correlated with a poorer prognosis in patients with HCC;[116] therefore, whether TIMs in irradiated tumors could subsequently reduce the efficacy of RT in HCC needs to be further investigated.

A combination treatment of irradiation with intravenous injection of recombinant macrophage inflammatory protein-1α (MIP-1α), the chemoattractant cytokine of monocytes, prevented lung metastasis and increased survival of murine hepatoma.[117] This was achieved by significantly increasing antitumor $CD11C^+$ dendritic cell infiltration into irradiated tumors. Nevertheless, the function of TAMs in this process was not mentioned. Zoledronic acid (ZA) has been found to revert TAM polarization from the M2 to M1 phenotype in some tumors;[118] however, it is still not clear whether or not this occurs in HCC. Previously, combined treatment of metastatic HCC with RT and ZA was reported to result in its unexpected regression.[119] This synergistic effect in anti-liver cancer may be associated with the decreased infiltration of TAMs and the adjusted immunological milieu afforded by ZA.[119,120] More recently, RT plus ZA treatment has been implicated in decreasing bone pain and improving overall survival in patients with bone metastases from HCC.[121] Indeed, combined treatment presented a changed TME, as shown by the reduced

levels of IL-6, lack of MIP-1α production and concurrent MMP-2, -3 and -9 downregulation.[121] Therefore, different HCC RT combination schemes may provide beneficial effects to the immune system and may improve clinical outcomes. Many preclinical studies elucidated a synergistic effect when RT and ICIs were combined, which became an evolving systemic therapy for HCC.[122] The increased level of soluble PD-L1 (sPD-L1) after RT correlated with HCC aggressiveness and outcomes, suggesting the role of RT plus ICIs as a possible intervention for HCC (Fig. 3).[123]

Hopefully, encouraging results of more preclinical and clinical trials incorporating RT alone or as a part of a combined treatment will be found and can provide novel therapeutic choices for HCC.

3. Immunotherapy

For a long time, advanced HCC has been a serious therapeutic challenge with limited treatment options. Intriguingly, immunotherapy alone or in combination with other systemic therapies is rapidly becoming a promising therapeutic approach for improving clinical benefits in HCC patients by providing effective control of hepatic immune cells,[16] including macrophages,[124] T cells,[125] and nature killer cells.[126] A variety of checkpoint molecules,[127] including PD-1/PD-L1, CTLA4, TIM3, LAG3 and TIGIT, mediate immunosuppression and progression of tumors by altering the status of immune surveillance and attenuating antitumor T cell responses.[128] Checkpoint inhibition immunotherapy, such as the application of anti-PD-1/PD-L1 antibodies as ICIs, has greatly improved clinical outcomes in patients with HCC.[129] However, the proportion of patients responding to this monotherapy is low due to the complexity of the HCC TME.[20,130] Therefore, a deeper understanding of biomarkers and targets is crucial for effectively predicting HCC patient responses to treatment and improving treatment efficacy. A decrease in C-C motif chemokine ligand 14 (CCL14) was negatively associated with the expression of PD-1, TIM-3 and CTLA-4 in HCC

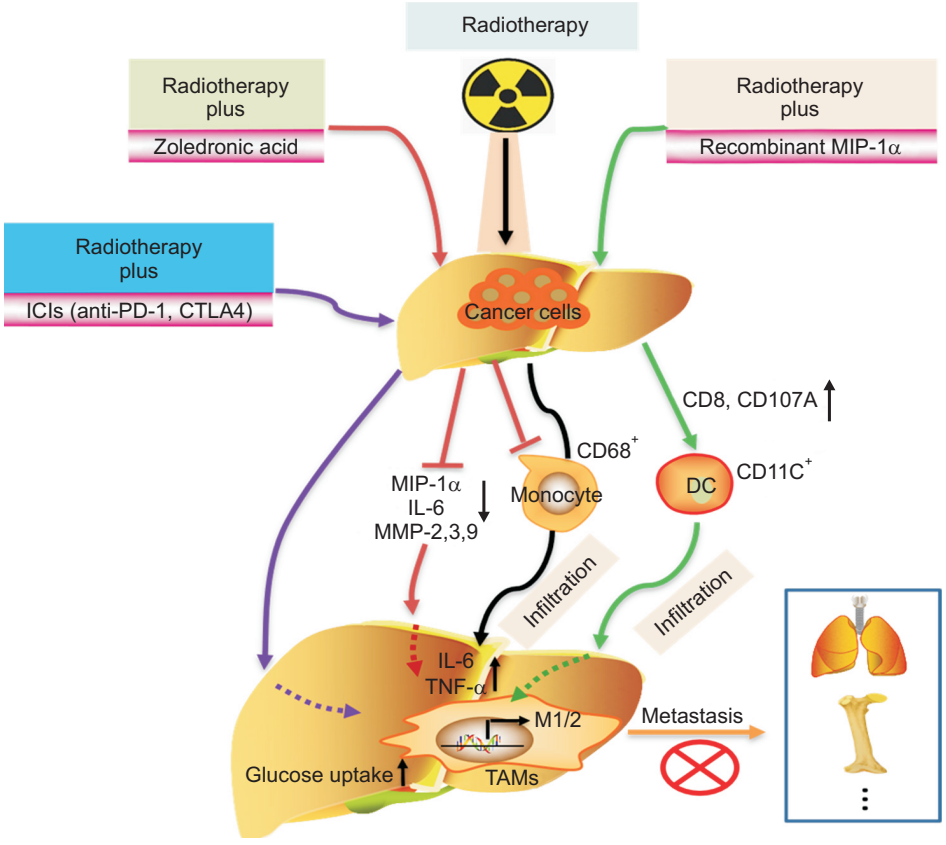

Fig. 3. Functional characterization of tumor-associated macrophages (TAMs) in hepatocellular carcinoma (HCC) radiotherapy. Irradiation in patients with HCC largely prevents tumor growth and causes a continuous influx of recruited F4/80^{+}CD68^{+} (M1) macrophages into the irradiated tumors. Emerging evidence suggests the efficacy of radiotherapy when used in combination with different existing therapies, including recombinant macrophage inflammatory protein-1 α (MIP-1α), zoledronic acid and immune checkpoint inhibitors (ICIs), for the treatment of metastatic HCC. These results show that targeting TAM infiltration and function potentiates the anti-liver cancer effects of radiotherapy.
CTLA4, cytotoxic lymphocyte antigen 4; IL, interleukin; MMP, matrix metalloproteinases; PD-1, programmed cell death protein 1; TNF-α, tumor necrosis factor-α.

and correlated with poorer prognosis; CCL14 mediated the infiltration of various tumor immune cells, including macrophages.[131] Strikingly, nanoliposome-loaded C6-ceremide injected into HCC mice could reduce the number of TAMs, which in turn promoted the antitumor immune response of $CD8^+$ T cells.[132] Advanced molecular techniques in paradigm-shifting studies have uncovered heterogeneous TAMs as critical regulators of the TME, and targeting TAMs might result in robust antitumor immune effects in HCC.

The immune checkpoint molecule PD-1 and its ligand PD-L1 are mainly expressed on the surface of immune effector cells and cancer cells, respectively.[133] Although it is well established that activation of the PD-1/PD-L1 pathway inactivates T cells and facilitates immune evasion, little is known about this pathway, which may have an important role in TAMs.[134] First, high $M2^+$ TAM density in the TME significantly elevated PD-L1 expression in esophageal cancer cell and was associated with shorter survival.[135] The authors of that study suggested that TAMs could function as a prognostic biomarker; however, whether macrophages might also express PD-1 in the TME was not mentioned. Several studies have recently confirmed that the expression of PD-1 on TAMs and $PD\text{-}1^+$ TAMs was negatively correlated with the prognosis of cancers due to decreased macrophage phagocytosis[134] and increased cancer cell invasion,[136] which occurred concomitantly with a predominantly M2 phenotype, which was different from the phenotype of PD-1–TAMs.[134,136] These results suggested that PD-1/PD-L1 therapies may function in a macrophage-dependent fashion, which has substantial implications for HCC treatment. As expected, endoplasmic reticulum stress-related exosomes derived from HCC cells activate the miR-23a-PTEN-AKT pathway and stimulate the upregulation of PD-L1 in macrophages, promoting tumor cell escape from antitumor immunity.[137] Deficiency of miR-148b in HCC cells activated the CSF1 pathway, which promoted TAM infiltration into the HCC microenvironment, leading to HCC progression and metastasis.[53] Moreover, the upregulation of TAM infiltration resulted in the overexpression of PD-L1 based on the NF-κB/STAT3 pathway in HCC cells.[124] Inhibition of the osteopontin/CSF1/CSF1R

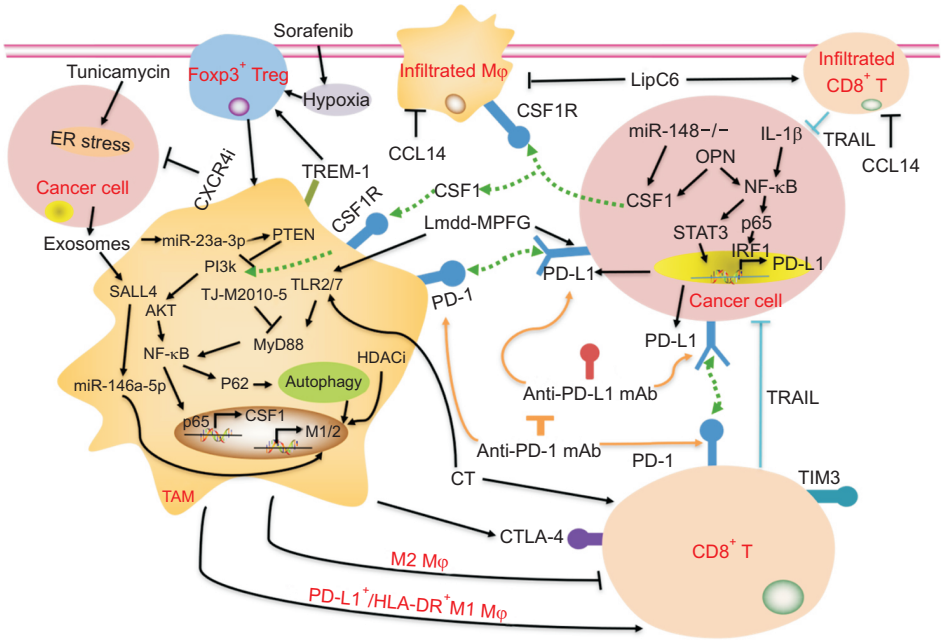

Fig. 4. Interactions among tumor-associated macrophages (TAMs) and other cellular components of the hepatocellular carcinoma (HCC) immune microenvironment during immunotherapy. Immunotherapies are rapidly becoming promising therapeutic approaches for patients with HCC. M2 TAMs largely contribute to the generation of an immunosuppressive environment by expressing multiple immune checkpoint molecules. Reprogramming TAMs from the M2 phenotype into the M1 phenotype might be a promising strategy for disrupting the immunotolerance-inducing mechanisms that occur during HCC immunotherapy.

AKT, protein kinase B (PKB); CXCR, CXC chemokine receptor; CSF1, colony stimulating factor-1; CSF1R, CSF1 receptor; CT, cryptotanshinone; CTLA-4, cytotoxic lymphocyte antigen 4; ER, endoplasmic reticulum; HDACi, HDAC inhibitor; HLA-DR, human leukocyte antigen-antigen D related; IRF, interferon regulatory factor; mAb, monoclonal antibodies; LipC6, nanoliposome-loaded C6-ceremide; Lmdd-MPFG, *Listeria monocytogenes*-based tumor vaccine; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor kappa B; OPN, osteopontin; PD-1, programmed cell death protein 1; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SALL4, Sal-like protein-4; STAT3, signal transducer and activator of transcription 3; TIM, tumor-infiltrating macrophage; TLR, Toll-like receptor; TREM-1, triggering receptor expressed on myeloid cells-1; TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand.

signaling pathway could reprogram TAMs from the M2 to M1 phenotype and inhibit PD-L1 expression, thereby enhancing the response to anti-PD-1/PD-L1 therapy in HCC mice.[138] Targeting the key regulators of TAM infiltration and polarization might serve as an immunotherapeutic option for HCC. For instance, *Listeria monocytogenes*-based tumor vaccine (Lmdd-MPFG) vaccination was believed to promote TAM reversal from M2 to M1 phenotypes through TLR2/myeloid differentiation factor 88 (MyD88)-dependent NF-κB activation and through p62-mediated autophagy pathway promotion, synergizing the effects of PD-1 antibody treatment in HCC immunotherapy.[20] Cryptotanshinone (CT), a novel natural product molecule, has shown powerful antitumor activity for curing Hepa1-6-bearing mice when used with anti-PD-L1.[139] Mechanistically, CT possessed the dual capacities of promoting antitumor M1 polarization via the TLR7/MyD88/NF-κB axis and inducing an antitumor $CD8^+$ T cell response.[139] The roles of MyD88 in TAMs may be different, which may cause M1 or M2 polarization in different contexts. It is conceivable that MyD88 might play a key role in the generation of pro-tumor immunity, and treatment with its inhibitor, TJ-M2010-5, resulted in an increase in the antitumor M1 macrophages (F4/80 CD11c) in the TME and decreased HCC growth.[140] Combining an anti-PD-1 immunotherapeutic agent, nivolumab, with an HDAC inhibitor could both improve the M1 polarization and antitumor activity of anti-PD-1 therapy.[141] An interesting study by Zong *et al.*[142] showed that infiltrated $CD68^+$ human leukocyte antigen-antigen D related $(HLA\text{-}DR)^+$ M1 macrophages triggered the expression of PD-L1 through activated IL-1β/p65/IRF1 pathway in HCC cells, and they concluded that there was a pro-tumor role for M1 TAMs. M1 macrophages expressed higher levels of PD-L1/HLA-DR than M2 macrophages.[142-144] Nevertheless, upregulated PD-LI in hepatoma cells caused by the Lmdd-MPFG vaccine might resensitize local tumor $CD8^+$ T cells to respond to anti-PD-1 immunotherapy, which would further induce M1 TAM polarization through the TLR2/MyD88 pathway.[20] More recently, $CD68^+CD11b^+$ (M1) macrophages were found to form an antigen-presenting niche to differentiate stem-like, tumor-specific $CD8^+$ T cells, which was required for maintaining a CD8 T cell response to human cancer.[145] These findings mean that PD-L1 expression in TAMs may be a favorable marker of anti-PD-1 treatment in HCC. Therefore, manipulation of TAM M1 polarization and/or depletion of M2 TAMs might offer a clinically relevant predictive/prognostic marker of response to ICI therapy.[146]

Apart from PD-1/PDL-1, other ICIs might also be involved in TAM-mediated HCC immunotherapies. HCC-derived exosomes contribute to cancer progression by promoting M2 polarization through the axis of the transcription factor Sal-like protein-4 (SALL4) and miR-146a-5p, by which PD-1 and CTLA-4 expression is increased in T cells.[48] Another study unveiled that $PD1^{Hi}$ $CD8^+$ depleted T cells in HCC highly expressed exhaustion-related inhibitory receptors (TIM3 and CTLA-4) and that these cells were in close proximity to PD-L1+TAMs; further, these observations correlated with poor prognoses for HCC patients.[147] The myeloid inhibitory immunoreceptor signal regulatory protein α interacts with cluster of differentiation 47 (CD47) and can be viewed as a primary regulatory "checkpoint" for macrophages, serving as a protective signal for escaping macrophage surveillance and phagocytic elimination in various cancers.[148] Antibody-mediated CD47-blocking immunotherapy alone[149] or in combination with doxorubicin exerted suppressive effects on HCC by inducing macrophage-mediated phagocytosis.[150] The triggering receptor expressed on myeloid cells-1 (TREM-1) is a novel receptor of the innate immune system that amplifies pro-inflammatory responses by myeloid cells,[151] including macrophages.[152] Increased expression of $TREM\text{-}1^+$ TAMs is abundant at advanced stages of HCC, which is crucial for HCC resistance to anti-PD-L1 therapy and immunosuppression in the hypoxic TME because it promotes $CCR6^+$ Foxp3 Treg accumulation.[153] Increased hypoxia after sorafenib treatment led to the accumulation of M2 TAMs and Tregs in HCC,[154] and it proved to be linked with immunosuppression and poor prognosis.[154,155] In response to sorafenib-related hypoxic and immunosuppressive microenvironments, anti-PD-1 immunotherapy showed efficacy only when there was concomitant supplementation with CXCR4 inhibitors (Fig. 4).[154]

Altogether, immunotherapies are emerging as the most promising approaches for HCC treatment, but this new frontier of TAM-related HCC immunobiology still needs further exploration.

CONCLUDING REMARKS

HCC is one of the deadliest diseases due to the complicated TME and the deficiencies of therapies. It is well known that multiple carcinogenic factors contribute to the complexity and heterogeneity of HCC. Currently, any single treatment regimen for HCC shows obvious limitations, including unsatisfactory efficacy and safety, while combined therapies could play a critical role in the treatment of HCC in the future. Therefore, finding the key targets and common nodes in combined therapies has become the most important approach to HCC research.[156] Interestingly,

TAM function and polarization have shown clinicopathological significance in predicting prognosis and promoting the therapeutic efficacy of HCC monotherapy and/or combination therapy.[157] Thus, it could be desirable to consider TAM-based therapy for the preferred treatment of patients with advanced-stage HCC. Hopefully, the mechanisms and factors that TAM-triggered initiation and progression of HCC could be extensively investigated in preclinical models and in the clinic, potentially promoting innovative approaches and precise treatment for patients with HCC.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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