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Immune checkpoint blockade in hepatocellular carcinoma: Current progress and future directions

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Abstract

Immune checkpoint blockade has recently emerged as a promising therapeutic approach for various malignancies including hepatocellular carcinoma (HCC). Preclinical and clinical studies have shown the potential benefit of modulating immunogenicity of HCC. In addition, recent advances in tumor immunology have broadened our understanding of the complex mechanism of immune evasion. In this review, we summarize the current knowledge on HCC immunology, and discuss the potential of immune checkpoint blockade as a novel HCC therapy from the basic, translational, and clinical perspectives.

Introduction

The failure of the immune system to prevent hepatocellular carcinoma (HCC) and to halt its progression is closely linked with pathogenesis and the survival of patients with HCC. Thus, immunotherapy aimed at boosting HCC-specific immune responses is considered a promising treatment approach. In this review, we will summarize the current knowledge of immune responses in HCC—with the focus on immune checkpoints—and the current status of translational clinical research in this field.

Why use immunotherapy for HCC patients?

The success of immune checkpoint blockade with anti-cytotoxic T lymphocyte associated antigen 4 (CTLA-4) antibodies in advanced melanoma patients has brought renewed hope for immunotherapy in cancer. In addition, immunotherapy is particularly attractive for HCC for several reasons. HCC is typically an inflammation-associated cancer and can be immunogenic. Indeed, cases of spontaneous regression of HCC have been reported and many of these cases were related to systemic inflammatory responses [1]. In addition, the majority of HCC patients suffer from cirrhosis of viral etiology, alcoholism, or nonalcoholic steatohepatitis. Since immunotherapeutic drugs are not metabolized in the liver, they may

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have predictable pharmacokinetic profiles in cirrhotic patients. Indeed, preliminary clinical data with antibody-based therapy has not shown any severe hepatotoxicity [2]. Nevertheless, the successful application of immunotherapy in HCC will have to take into account the liver cancer-specific immune microenvironment and responses.

How do HCCs evade anti-tumor immunity?

Spontaneous anti-tumor responses have been detected in HCC patients. Activation of immune response and T cell infiltration has been reported after percutaneous ethanol injection and radiofrequency ablation (RFA) [3,4]. In addition, tumor-associated antigen (TAA) specific CD8+ T cell immune responses have been described. Among the most studied antigens in HCC are alpha-fetoprotein (AFP), glypican-3 (GPC-3), NY-ESO-1, SSX-2, melanoma antigen gene-A (MAGE-A) and human telomerase-reverse transcriptase (hTERT). One report estimated that more than 50% of HCC patients develop spontaneous cellular or humoral immune response against NY-ESO-1 [5]. Another study reported that HCC-infiltrating TAA-specific CD8+ T cells were detectable in more than 50% of patients, and these cell numbers correlated with progression-free survival [6].

The immune microenvironment of the liver plays a major role in anti-tumor immunity. Liver is generally “tolerogenic” to prevent undesirable immune response to antigens absorbed from the gut. The tolerability is maintained by direct activation of naïve T cells in liver through antigen presentation by liver sinusoidal endothelial cells, Kupffer cells, dendritic cells (DCs) and hepatocytes [7–10]. In addition, intricate immunosuppressive mechanisms become activated in the HCC microenvironment and further interfere with the development of meaningful anti-tumor immune responses. Multiple such mechanisms have been proposed, including defective antigen presentation, recruitment of immunosuppressive myeloid and lymphoid cell populations, suppression of natural killer (NK) cells, impaired CD4+ T cell functions, and up-regulation of immune checkpoint pathways [11–23].

Among immunosuppressive cell populations, T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs) are thought to play key roles in cancer evasion from immunosurveillance. In HCC, the number of Tregs is increased both in the blood circulation and inside the tumor [11]. Intratumoral Treg accumulation correlates with disease progression and poor prognosis [12, 13]. MDSCs are immature/progenitor myeloid cells with immunosuppressive and pro-angiogenic activity. MDSC accumulation is found not only within the tumors but also in blood circulation, spleen, bone marrow and liver [14]. The MDSCs inhibit the function of effector T cells, and decrease NK cell cytotoxicity and cytokine production [15, 16]. The frequency of MDSCs has been shown to correlate with recurrence-free survival of HCC patients who have undergone RFA [17]. It has also been suggested that MDSCs interact with Kupffer cells to induce PD-L1 expression, which in turn inhibits antigen presentation [14]. MDSCs may also help expand Treg population. Depletion of Tregs or MDSCs could prompt spontaneous immune responses against AFP, suggesting the potential of immune reactivation [15, 23]. Recently, Han et al. have identified new subset of immune suppressive cells in HCC patients called regulatory DCs [24]. These regulatory DCs can suppress T cell activation through interleukin (IL)-10 and indoleamine 2,3-dioxygenase (IDO) production.

Exhaustion of CD4⁺ T cells has also been reported as a mechanism of immune evasion in HCC. While infrequent AFP-specific CD4⁺ T cells are detectable in early disease, they became exhausted and fail to execute their immune supportive function once the disease has advanced [18, 19].

Finally, while the immune response to specific antigen is recognized by major histocompatibility receptors, co-stimulatory and co-inhibitory molecules regulate the intensity of response. Immune checkpoints are co-inhibitory molecules that are physiologically expressed for the maintenance of self-tolerance. In the tumor microenvironment, immune checkpoint molecules such as CTLA-4 and PD-L1 are often overexpressed and participate in the evasive mechanism as discussed above [25–29]. In the next section, we will discuss the exciting potential of blocking the immune checkpoint for HCC therapy.

Immune checkpoint blockade: Potential for cancer immunotherapy

The balance between co-stimulatory signals and immune checkpoints determines the cytotoxic T cell activation and intensity of immune response [30]. The immune checkpoints are often activated in the tumor tissue, and this promotes tumor evasion from host immunity. The most studied immune checkpoint receptors are CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3 and OX40 (Fig.1 &Suppl. Table S1). Inhibitors of CTLA-4 and PD-1 have already been FDA-approved for the treatment of melanoma and are currently in development in HCC. Our review focuses primarily on the potential of these two pathways as targets in HCC.

CTLA-4: Mechanism of action

Although the mechanism of action of CTLA-4 (also known as CD152) is not fully elucidated, it is clear that this immune checkpoint acts as a “break” for immune responses. CTLA-4 is expressed on activated T cells and Tregs, and, at low levels, may also be expressed on naïve T cells [31–33]. Upon stimulation via the T cell receptor, CTLA-4 localizes to the plasma membrane. CTLA-4 can bind to CD80 and CD86 with much higher affinity than CD28 [34]. In doing so, CTLA-4 antagonizes the CD28 binding to CD80 and CD86 and inhibits T cell activation. CTLA-4 is known to inhibit the binding of antigen presentation by antigen-presenting cell (APC) [35]. Reverse signaling through CD80 or CD86 activates IDO in APC, which leads to tryptophan degradation and suppression of T cell-mediated anti-tumor immune responses [36]. CTLA-4 signaling may stimulate the expression of immune regulatory cytokines such as TGF-beta [37]. In addition, inhibition of CD28 interaction with CD80 or CD86 binding in APCs results in reduced T cell activation. Knockout of CTLA-4 is embryonically lethal in mice due to autoimmune response and excessive CD4⁺ T cell proliferation, suggesting that CTLA-4 function is primarily important in CD4⁺ T cells [38]. Although the exact mechanism is not known, CTLA-4 is also thought to have an effect on the differentiation and activation of Tregs, which constitutively express this receptor. Treg-specific knockout or blockade of CTLA-4 inhibits their ability to regulate both autoimmunity and anti-cancer immunity [39, 40].

Despite this understanding, and the promising results obtained so far with CTLA-4 inhibitors in clinical trials, mechanistic data on CTLA-4 blockade from preclinical models of HCC are limited. CTLA-4 blockade combined with microwave ablation and local GM-CSF administration showed promising efficacy in subcutaneous models of HCC [20]. Re-challenging the mice by re-implantation of the cancer cells resulted in tumor rejection in 90% of the cases. Anti-tumor responses by CD4⁺ and CD8⁺ T cells and NK cells in the circulating blood were also observed, suggesting successful immunization by this strategy. Future studies in more clinically relevant models of HCC should provide mechanistic insights to guide clinical development.

Clinical studies of CTLA-4 blockade

Two CTLA-4 blocking antibodies (ipilimumab and tremelimumab) are currently in advanced stages of clinical development (Table 1). The US FDA approved ipilimumab for the treatment of melanoma in 2011 based on randomized phase III trial showing improved overall survival [41, 42]. Recently, tremelimumab showed early evidence of antitumor activity in a single arm phase II trial in malignant mesothelioma [43]. CTLA-4 blockade has also been tested in other malignancies, including breast, colon, lung, prostate and brain cancers, melanoma, lymphoma and sarcomas. Two observations are consistent in the published studies [44]. Firstly, the efficacy of CTLA-4 blockade may correlate with the immunogenicity of the tumor. Secondly, the immunotherapy may be more effective against smaller tumors.

A phase I trial of tremelimumab in HCC patients has recently been reported (NCT01008358) [43]. The study enrolled 21 patients with chronic hepatitis C with Child-Pugh A or B cirrhosis and advanced HCC not amenable to percutaneous ablation or transarterial embolization. Tremelimumab was well tolerated, and there were no treatment related deaths. Almost half of the patients experienced a grade 3/4 rise in AST but this was not associated with a parallel decline in liver dysfunction. Partial responses were seen in 17.6% of the cases, and 45% of the patients had stable disease for more than 6 months. Interestingly, the patients with stable levels of interferon (IFN)- γ during the treatment showed better treatment response compared to those who showed a decrease, suggestive of more active anti-tumor immunity. A phase I clinical trial of tremelimumab with radiofrequency ablation or transarterial therapy is ongoing [NCT01853618].

PD-1: Mechanism of action

PD-1 is another CD28 superfamily member that conveys co-inhibitory signals for TCR receptor. PD-1 binds to its ligands PD-L1 (CD274) or PD-L2 (CD273) [45, 46]. PD-1 is primarily expressed on CD8⁺ T cells, but can also be detected on Tregs and MDSCs [47]. PD-1 mediates the differentiation and proliferation of Tregs [48]. PD-1 also regulates peripheral tolerance and autoimmunity. Chronic exposure to antigens leads to the overexpression of PD-1 in T cells, which induces anergy or cell exhaustion [49]. By chronic antigen stimulation, IFN- γ induces IRF9 binding to Pdcd-1 promoter and PD-1 transcription in T cells. When PD-1 binds to PD-L1 or PD-L2, T cell proliferation and cytokine release are inhibited through SHP2, which inactivates ZAP70, a major TCR signaling integrator

[50]. T cell function is differentially affected by the level of PD-1 activity [51]. Cancer cells can hijack PD-L1/PD-1 signaling by expressing PD-L1 or PD-L2 to activate PD-1 in tumor-infiltrating lymphocytes and evade immune surveillance [52, 53].

While the mechanisms of immune tolerance to viral hepatitis are well described, limited data are available for HCC [54, 55]. Two mouse models showed the potential relevance of PD-1/PD-L1-induced immune tolerance in HCC. In a genetic model of c-Myc-induced HCC and doxycycline-induced expression of IL-12 in hepatocytes, doxycycline treatment induced IFN- γ expression but only a partial regression of the tumors [21]. Treatment resistance was associated with an increase in Treg numbers and upregulation of several immune checkpoint molecules (including PD-L1/PD-1). In another mouse model of HCC induced by adenovirus-mediated inducible SV40 large T antigen expression in hepatocytes, T cell infiltration into the tumor was found to be decreased in the advanced lesions [22].

Translational clinical studies also support the potential role of PD-1/PD-L1 pathway-induced immune tolerance in HCC. PD-L1 was found to be expressed by cancer cells and PD-1 by CD8⁺ T cells in HCC tissue specimens [25]. Moreover, correlative studies have shown that both PD-L1 expression and PD-1 expression in tumors were significantly correlated with HCC stage, local recurrence rate and poor prognosis [26, 56–58]. Similarly, the frequency of intratumoral or circulating PD-1⁺CD8⁺ T cells correlated with HCC progression and postoperative recurrence [27].

Even in cases when CD8⁺ T lymphocytes were activated and recognized the tumor (as evidenced by HLA-1 expression), high PD-L1 expression negatively impacted prognosis in resected HCC patients [56]. Furthermore, PD-L1 and PD-1 expression in circulating cells correlated with poor prognosis in HBV-positive HCC patients who underwent cryoablation [28]. Finally, simultaneous depletion of Tregs, MDSC and PD-1⁺ T cells from the peripheral blood of advanced HCC patients could restore CD8⁺ T cell activation *ex vivo* [29].

Clinical studies of PD-1 blockade

Five anti-PD-1 antibodies and 3 anti-PD-L1 antibodies are currently under development, emphasizing the growing interest in this immune checkpoint pathway as a target for cancer therapy (Table 1). Lambrolizumab induced tumor regression in advanced melanoma patients and showed a favorable safety profile [59]. Interestingly, lambrolizumab was effective even in patients who failed ipilimumab treatment, which suggests a differential mechanism of action for PD-1 inhibition versus CTLA-4 blockade. Indeed, combination of nivolumab with ipilimumab achieved objective response in 40% of the patients with limited toxicity [60]. CT-011 and MPDL3280A/RG7446 were tested in phase I trials and showed with favorable safety profiles [61]. MEDI4376 targets PD-L1, and phase I trial is ongoing. AMP-224 is a recombinant B7-DC-Fc fusion protein, and a phase I trial of this agent is also underway. In HCC, a phase I/II trial of CT-011 in advanced HCC was initiated but stopped due to slow accrual. A phase I trial of nivolumab is currently ongoing for patients with advanced HCC (NCT01658878). This study plans to enroll three cohorts of patients stratified by viral etiology—HCV, HBV, no viral infection—with a target enrollment of 90 patients.

Future perspectives

Immunotherapy using immune checkpoint blockers has already shown great promise in intractable cancers such as advanced melanoma. This approach has recently begun clinical testing in advanced HCC patients. The limited evidence from pre-clinical studies in animal models further supports the development of CTLA-4 and PD-L1/PD-1 inhibitors in this disease. Successful development of this strategy will have to address several aspects specific to HCC. Firstly, it will need to address the specific issues of immunotolerance and chronic liver inflammation (cirrhosis) and hypoxia that underlie HCC development and response to treatment [62]. Secondly, the strategy will be most likely to succeed if the development will be guided by biology-driven biomarkers of response to such agents. Thirdly, therapy with immune checkpoint blockers is most likely to succeed in combination with other surgical, cytotoxic, immune or targeted therapies. Combination of immune checkpoint inhibitors with local ablative therapies such as RFA or cryoablation—which may induce tumor antigen release—would particularly promising approaches [63]. Furthermore, integration of immunotherapy with systemic treatments (e.g., sorafenib) will require mechanistic understanding of treatment interactions. Finally, the integration of immune checkpoint blockers—alone or in combination with other agents—will have to address the safety concerns specific to this population of patients such as hepatotoxicity. The potential risk of fueling acute exacerbation of viral hepatitis in HBV/HCV positive patients will need to be elucidated and carefully observed in the clinical setting. Addressing these issues will greatly help the field bring to fruition the great promise of these novel immune-therapeutics in this intractable disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Role of immune checkpoint molecules in anti-tumor immune responses against hepatocellular carcinoma

(a) Cancer cells enhance the intratumoral recruitment and expansion of MDSCs and Tregs. Tregs and MDSCs suppress innate immune response of NK cells through activation of immune checkpoint pathways such as PD-L1/PD-1. MDSC or Treg activity is regulated by the inflammatory tumor microenvironment, helper T cells or dendritic cell activities. (b) The acquired immune responses are suppressed through multiple mechanisms by immune checkpoint molecules. Cytotoxic T cell function is not only subdued by the immune suppressive cells such as Tregs and MDSCs but also repressed by the co-stimulation of CTLA-4 with antigen presentation from DCs. The tolerogenic liver microenvironment also inhibits cytotoxic T cell activation.

Table 1

Immune checkpoint blocking antibodies and status of clinical development for HCC

Target	Antibody	Trial ID	Phase	Treatment	Status	Results
CTLA-4	Tremelimumab(formerly referred to asticilimumab, CP-675,206, MedImmune, USA & Pfizer, USA)	NCT01008358	I	Monotherapy	Completed	Well tolerated Disease control rate of 76.4% Median OS 8.2 month [95%CI: 4.64,21.34]
		NCT01853618	I	In combination with RFA or TACE	Ongoing	N/A
		N/A	N/A	N/A	N/A	N/A
PD-1	Ipilimumab(MDX-010, Bristol-Myers Squibb, USA)	NCT01658878	I	Monotherapy	Ongoing	N/A
			I/II	Monotherapy	Terminated	Stopped due to slow accrual
			N/A	N/A	N/A	N/A
			N/A	N/A	N/A	N/A
			N/A	N/A	N/A	N/A
PD-L1	MPDL3280A/RG7446 (Genentech, USA &Roche, Switzerland) MED14376 (MedImmune, USA &AstraZeneca, UK)	N/A	N/A	N/A	N/A	N/A
		N/A	N/A	N/A	N/A	N/A