Program death-1 immune checkpoint and tumor microenvironment in malignant liver tumors

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Abstract

Hepatic malignancies are one of the leading causes of cancer death globally. Considering the limited efficacy of current standard treatments in management of patients with advanced liver cancers, there has been a growing interest in identifying novel therapies. Despite achieving promising results in initial clinical trials, the therapeutic benefit of immunotherapy is limited due to strong immune-tolerogenic characteristics of liver tumors. Therapeutic regimens that impede tumor immunosuppressive mechanisms or elaborate tumor-specific immunity may improve clinical outcomes of patients with liver malignancies. Programmed cell death 1 (PD-1), an inhibitory checkpoint molecule, and its ligands (PD-L1 and -L2) are the main mediators of immunosuppression within the tumor microenvironment. The expression level of PD-1/PD-L1 may act as a biomarker to predict disease progression, as well as long-term survival. Furthermore, early trials have demonstrated the efficacy and safety of targeting PD-1/PD-L1 as an emerging field in the management of patients with advanced hepatocellular carcinoma. We herein review the role of PD-1/PD-L1 in the pathogenesis of liver malignancies, as well as its potential diagnostic and therapeutic implications.

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1. Introduction

Liver cancer is the sixth most common cancer and third leading cause of cancer death globally. Liver malignancies encompass a
wide spectrum of diseases from primary liver cancers such as hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) to metastatic cancers. Similar to other cancers, liver tumors harbor numerous genetic and epigenetic alterations, which can lead to the production of neoantigens. These neoantigens are recognizable by the immune system and can potentially elicit an endogenous immune response (Fig. 1) [1,2]. Tumors may, however, evade immune destruction through development of several resistance mechanisms including local immune suppression, induction of immune cell tolerance, and systemic dysfunction in T-cell signaling [3,4].

Targeting immune checkpoints with small molecules might either turn up (co-stimulatory checkpoint molecules) or turn down (co-inhibitory checkpoint molecules) T-cell signaling. In recent years, inhibitory checkpoint molecules have specifically been investigated as novel targets for cancer immunotherapies (Table 1). One of the most established drugs of this category, Ipilimumab (Yervoy, from Bristol-Myers Squibb), is a monoclonal antibody that inhibits CTLA-4 (an inhibitory checkpoint molecule). In 2011, Ipilimumab gained United Stated Food and Drug Administration (FDA) approval for treatment of patients with advanced melanoma [5,6].

Programmed cell death 1 (PD-1), another inhibitory checkpoint molecule, and its ligand (PD-L1) have been identified as critical mediators of immunosuppression within the tumor microenvironment (Fig. 2) [7,8]. Several studies have demonstrated the safety and efficacy of anti–PD-1 and –PD-L1 monoclonal antibodies in the management of patients with advanced cancer [9,10]. We herein review the role of PD-1/PD-L1 in the pathogenesis of liver malignancies, as well as its potential diagnostic and therapeutic implications.

2. Mechanisms of immunologic tolerance in the liver

Interferon-gamma (IFN-γ) is an important pro-inflammatory cytokine, which controls production of several downstream effector molecules including CD-40, CD-86, and PD-L1 [11,12]. PD-L1 expressed by non-parenchymal cells in response to IFN-γ causes immune tolerance by binding to resident T-cells thereby causing apoptosis [13,14]. Furthermore, the population of immune cells within the liver can recruit myeloid-derived suppressor cells (MDSCs) to the microenvironment. MDSCs following interaction with hepatic stellate cells (HSCs) contribute to immune suppression through inhibition of T-cell (both CD4+ and CD8+ T-cells) proliferation [15–17]. Via a separate mechanism, HSCs in the presence of IFN-γ mediate immune tolerance through the generation of T-regulatory (Tregs) cells [18]. Selective depletion of Tregs abrogates tolerance by concomitant increases in CD4+ and CD8+ T-cell responses and reduced apoptosis of infiltrating T-cells [19,20]. Additionally, HSCs suppress the local T-cell response and contribute to migration and invasion of HCC tumor cells via overexpression of

![Fig. 1. The major steps of generation of immunity against cancer, which ultimately result in cancer cell death. CTLs – cytotoxic T lymphocytes.](image_url)

Table 1

<table>
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<tr>
<th>Target cell</th>
<th>Target molecule</th>
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<th>Commercial name</th>
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Ono — Ono Pharmaceutical Co., Ltd.; BMS—Bristol-Myers Squibb.
PD-L1 [21]. Blockade of PD-1/PD-L1 interaction is associated with significant reduction of HSC immuno-modulatory activity [21].

3. PD-1/PD-L1 and hepatitis

T-cells antiviral functions are impaired in patients with chronic hepatitis B (HBV) and hepatitis C virus (HCV) infections. In contrast to patients with asymptomatic HBV-infection, the frequencies of HBV-specific CD8⁺ T cells are similar among patients with active chronic HBV infection (CHBV) and HBV-induced HCC [22]. However, patients with active CHBV and HBV-HCC have significantly reduced HBV-specific CD8⁺ T-cell response after HBV peptide stimulation. This has been attributed to the decreased frequency of PD-1⁺ TIM-3⁺ double-negative T-cells, the main contributor to HBV-specific inflammation [22]. In a study by Xu et al., CD4⁺ and CD8⁺ T-cell PD-1 expression was increased in both patients with CHBV, as well as cirrhosis/HBV–HCC. Of note, among patients with CHBV, PD-1 expression was related to progression of the HBV infection, HBV viral load, and liver function [23].

Halting co-inhibitory pathways such as PD-1/PD-L1 may increase the function of T-cells (Fig. 3) [24–26]. In a study of patients with chronic HBV and HCV infection, Fisicaro et al. reported that stimulation of CD137 alone or in combination with anti-PD-L1 antibodies increased the responsiveness of intrahepatic T cells to HBV [27]. Although a similar effect was not observed in intrahepatic T cells of HCV patients, peripheral blood HCV-specific T cells demonstrated an augmented response. Although not significant, HBV-infected livers had a higher proportion of CD4⁺ regulatory lymphocytes compared with livers from patients who had chronic HCV infection. Furthermore, CD4⁺ cells from patients with chronic HBV infection had higher levels of PD-1 compared with patients who had chronic HCV infection (P = 0.009) [27].

4. PD-1/PD-L1 and HCC

4.1. Animal models

In murine liver tissue, PD-L1 is expressed by Kupffer cells (KCs) and liver sinusoidal epithelial cells (LSECs) [28]. PD-L1 deficiency has been demonstrated to lead to hepatic accumulation of T-cells due to impaired apoptosis and enhanced proliferation of effector cells during adenoviral infection [29,30]. T-cells represent the main mechanism of surveillance against virus-associated tumors, primarily due to the tumors high immunogenicity. The reason that surveillance occasionally fails, especially in the case of HBV- or HCV-associated HCC, is not completely understood. In a murine
model of HCC generated from activation of Simian Vacuolating Virus 40 T antigen (SV40 large TAg) with hepatocyte-specific adenovirus infection, Willimsky et al. reported that most of the infected cells were cleared by cytotoxic T lymphocytes [31]. Some of the virus-infected cells with lower immunogenicity were, however, able to escape immune clearance and progress to HCC. Furthermore, mice with advanced HCC had lower T cell infiltration compared with mice that had early stage tumors, suggesting a role of local immune suppression in the progression of HCC. The PD-1/ PD-L1 checkpoint pathway was proposed as one of the main mechanisms of local immune tolerance in this model [31]. In a different study, Li et al. established an animal model of HCC via intraperitoneal injection of carbon tetrachloride and intra-splenic inoculation of oncogenic hepatocytes [32]. In this study, upregulation of PD-1 on CD8\(^+\) T cells and accumulation of Tregs were two principal mechanisms of immune tolerance and hence tumor progression [32].

Pre-clinical models have identified PD-1/PD-L1 interaction blockade as a potential target for treatment of liver cancers. Blockade of PD-L1 with soluble PD-1 (sPD-1) has been associated with improved antitumor activity in vitro and in vivo [33]. Li et al. demonstrated that the combination of anti-PD-1 antibody with sumbiv marked suppressed tumor growth in a murine model of HCC [32]. The growth of HCC in mice that received combination therapy was less than mice treated with either medication alone. Simultaneous administration of the TLR3 agonist lysine-stabilized polyinosinic-polycytidylic acid (poly-ICLC) with sorafenib also was associated with improved HCC tumor control [34]. Restoring tumor immunogenicity through suppression of the co-inhibitory PD-1/PD-L1 signaling pathway, as well as augmentation of local immunity, contributed to the beneficial anti-tumor efficacy of combination therapy [34]. In contrast, administration of agents promoting PD-1/PD-L expression may be associated with poor prognosis. For example, the long-term use of indomethacin has been demonstrated to increase recurrence, as well as intrahepatic and distant metastasis of HCC through enhancing the expression of PD-1 and PD-L2 and reduction of TNF-\(\alpha\) and IFN-\(\gamma\) [35]. Zhao et al. reported that IL-17 contributed to immune escape among HCC tumors via induction of PD-L1 expression on monocytes in the peri-tumoral stroma [36].

Tumor cell immune resistance and changes in the microenvironment may be responsible, in part, for the failure of traditional cytotoxic chemotherapy. For example, Qin et al. reported that cisplatin up-regulated the expression of the immunoresistance molecule PD-L1 on hepatoma H22 cells, a potential mechanism of immune escape and cisplatin-resistance [37]. Finally, Woller et al. reported that oncolytic viral infection of malignant tumors abrogated resistance to systemic PD-1-immunotherapy [38]. Specifically, combined localized virotherapy and systemic PD-1 blockade was associated with more effective elimination of lung metastasis versus either therapy alone [38]. Upregulation of PD-L1 on tumor cells and the stimulation of a broad-range T-cell attack against the neoantigenome were recognized as potential mechanisms.

4.2. \textit{Human trials}

Tumor-induced immune suppression is one of the principal mechanisms that allows a tumors to proliferate and evade the immune system. Among patients with advanced HCC, immune dysregulation is characterized by augmented numbers of PD-1\(^+\)-exhausted T-cells, Tregs, myeloid-derived suppressor cells (MDSC), and increased levels of immunosuppressive cytokines (Fig. 4) [39,40]. Cytotoxic CD8\(^+\) T-cells represent the main effector cells in HCC and their inhibition through PD-1/PD-L1 pathway correlates with the progression of disease. Shi et al. reported that the frequency of circulating PD-1\(^+\) CD8\(^+\) T-cells, as well as the PD-1 expression level of tumor-infiltrating CD8\(^+\) T-cells was increased among patients who had progression of hepatic cirrhosis to HCC [41]. Moreover, Kalathil et al. noted that the percentage of PD-1\(^+\) CD4\(^+\) T-cells, as well as PD-1 expression levels, were much higher among patients with HCC patients versus healthy controls [39].

Despite the well-known inhibitory effects of PD-1 expression on T-cells, the role of PD-1/PD-L1 expression on myeloid cells including dendritic cells (DCs) and neutrophils has been not well-described. PD-1 has been demonstrated to be expressed on peripheral blood DCs and CD11c\(^+\) tumor-infiltrating myeloid cells of HCC patients compared with healthy controls [42]. In addition, expression of PD-1 on DCs was associated with the suppression of T cell response in vitro and in vivo. Furthermore, intratumoral transfer of PD-1-deficient DCs resulted in growth suppression of HCC tumors via promotion of tumor-infiltrating CD8\(^+\) T-cells and secretion of perforin and granzyme B [42]. Likewise, the predominance of intratumoural and peritumoral PD-L1 expressing neutrophils, as well as PD-L1 expression level of circulating neutrophils, were higher among HCC patients compared with healthy donors [43]. The PD-L1\(^+\) neutrophils suppressed the proliferation and activation of T-cells, which could be partially reversed by the blockade of PD-1/L1 [43]. The higher neutrophil-to-T-cell ratio in peritumoral tissue was also adversely related to postoperative overall survival (OS) [43].

PD-L1 expressing-monocytes mediated HCC tumor angiogenesis and aggressive growth by modulating the function of IL-22 producing T-helper cells (Th22 cells) [44]. Th22 cells seemed to participate in the inflammatory process of many diseases. Classical Th22 cells act independently of IFN-\(\gamma\) and IL-17, whereas the non-classical Th22 subset co-expressed IFN-\(\gamma\) and/or IL-17 along with IL-22 [45]. Of note, the non-classical Th22 subset represented the majority of Th22 cells in human liver and HCC tissues, while classical cells were predominant in blood [44]. PD-L1-expressing monocytes inhibited Th22 cell production of IFN-\(\gamma\), but increased IL-17 via interaction with PD-1. This phenomenon seemed to promote aggressive cancer growth and angiogenesis in vivo. These data further suggested that targeting PD-1/PD-L1 may be a potential anticancer strategy through modulation of this group of inflammatory cells [44]. Wu et al. examined human HCC specimens and demonstrated an increased predominance of PD-L1\(^+\) Kupffer cells (KCs) and PD-1\(^+\) CD8\(^+\) T-cells in tumor tissue versus the surrounding non-tumor tissue [46]. Furthermore, the expression of PD-L1 on HCC KCs was higher than the surrounding non-tumor as well as the normal liver tissue. In addition, the higher level of PD-L1 expression was correlated with a worse survival. On further analysis, the proliferative ability and function of PD-1\(^+\) CD8\(^+\) T-cells were noted to be impaired compared with the PD-1\(^-\) T-cells. The characteristics of effector T-cells were also reversed by blocking the KC PD-L1 interaction with PD-1\(^-\) CD8\(^+\) T-cells [46]. In a different study, Gao et al. reported that a higher expression of PD-L1 1 and -2 among HCC patients who underwent curative surgery was associated with a poor prognosis [47]. On multivariate analysis, PD-L1 was an independent predictor of postoperative recurrence, suggesting PD-L1 status as a novel predictor of recurrence and prognosis following surgical resection [47]. In a study of 141 patients with HBC-related HCC, Zeng et al. reported that circulating PD-1/PD-L1 expression was associated with HCC size, staging and invasion of blood vessels [48]. Moreover, patients with increased levels of PD-1/PD-L1 expression had a higher incidence of tumor recurrence and progression following cryoablation compared with patients in the low PD-1/PD-L1 group. Circulating PD-L1 has also been suggested to be an independent predictor of post-ablation recurrence-free and OS [48]. Furthermore, elevated levels of PD-1\(^+\) tumor-infiltrating...
lymphocytes have been associated with an increased prevalence of tumor thrombosis invasion into the portal vein [49].

5. PD-1/PD-L1 and other hepatic malignancies

In addition to HCC, the PD-1/PD-L1 pathway has been implicated in the pathogenesis of other hepatic malignancies including intrahepatic cholangiocarcinoma (iCCA) and colorectal liver metastasis (CRLM) [9,10,50]. In a study of 54 patients with iCCA who underwent resection, Gani et al. reported that 34 and 39 specimens were positive for PD-L1 expression on tumor-associated macrophages (TAMs) and cells within the tumor front (TF), respectively [50]. The expression of PD-L1 within the TF was associated with a 59.5% reduced survival ($P = 0.005$), although TF+ patients were less likely to present with lymph node metastasis (26.7% vs. 7.7% in TF- patients, respectively; $P = 0.011$) [50]. In a different study of 58 patients with iCCA, Fontugne et al. reported that PD-L1 expression on neoplastic cells and inflammatory cell aggregates were observed in 5 and 31 patients, respectively [51]. PD-L1 expression was associated with high density of CD3+ tumor-infiltrating lymphocytes. The authors concluded that the presence of a dense intratumoral lymphocytic infiltration might help to guide PD-1/PD-L1 blockade treatment in iCCA patients [51].

PD-1/PD-L1 has also been reported to be overexpressed in metastatic colorectal liver lesions, with stromal PD-L1 expression being associated with worse progression free survival [42]. In turn, PD-1/PD-L1 has been proposed as a potential therapeutic target for patients with CRLM. In animal models, down regulation of PD-1 within the tumor microenvironment by Listeria monocytogenes and the subsequent tumor-specific cytotoxic CD8+ T-cell response has formed the basis for the development of novel CRLM vaccines [52]. Interestingly, in one study of patients with synchronous CRLM who underwent primary tumor resection with or without preoperative ablation, ablation was associated with an increased T-cell infiltration, as well as PD-L1 expression [53]. In a mouse model, ablation led to a strong T-cell-mediated immune response, which was quickly reversed by inhibition of CD8+ and CD4+ T-cells function and up-regulation of PD-L1/PD-1 expression. In turn, combined treatment with ablation and anti-PD-1 antibodies resulted in enhanced T-cell immune responses and strengthened antitumor immunity with prolonged survival [53]. In separate murine model of carcinoembryonic antigen + (CEA+) liver metastasis, Burga et al. reported that liver MDSCs utilized the PD-1/PD-L1 pathway to suppress the anti-tumor responses induced by anti-CEA chimeric antigen receptor modified T cell (CAR-T) [54]. Of note, the efficacy of CAR-T immunotherapy was increased by administration of PD-L1 blocking agents.

6. PD-1/PD-L1 associated biomarkers

In addition to PD-1/PD-L1, other immune-related factors may be useful as biomarkers. For example, neoplastic cells may evade immune surveillance through down regulation of human leukocyte antigens class I (HLA class I). In a study of 80 patients who underwent resection, HLA class I expression was associated with a better recurrence-free survival (RFS), but no difference in OS. Similar to other studies, low expression of PD-L1 was associated with better OS, however the difference was not significant. The authors suggested that the combination of low PD-L1/high HLA class I on HCC tumors may be predictive of an improved recurrence-free survival and OS [55].

The composition of immune cells in the tumor microenvironment, the so called “Immunoscore,” has also been suggested to predict postoperative survival of patients [56]. Gabrielson et al. reported that high densities of CD3+ and CD8+ T-cells in both the interior and margin of resected HCC specimens were associated with a lower ($P = 0.007$) and a prolonged recurrence-free survival ($P = 0.002$) [57]. The expression of PD-L1 was also linked to lower recurrence ($P = 0.034$), as well as prolonged recurrence-free survival ($P = 0.029$) [57]. The authors concluded that both PD-L1 expression and Immunoscore may be important prognostic markers for HCC patients undergoing surgical resection.

Circulating soluble PD-1 (sPD-1) levels have also been associated with the risk of progression to HCC in patients with chronic HBV infection. In a study of 2903 men with chronic HBV infection, patients with increased sPD-1 plasma levels maintained a higher
viral load for 4 or more years. The combination of both high sPD-1 and viral load was associated with a 6.29-fold increase in the risk of HCC development. Interestingly, HBV genotype C increased the risk of HCC even more (odds ratio: 30.47) [58].

7. PD-1/PD-L1 inhibition as an adjunct to sorafenib for advanced liver cancer

Considering the limited therapeutic options for patients with advanced HCC, immunotherapy might serve as an adjunct therapy to available treatments. Sorafenib, a multi-kinase inhibitor, is a systemic treatment option for patients with advanced HCC. In addition to its anti-angiogenic effects (via VEGFR inhibition), sorafenib may enhance antitumor immunity [59]. In a murine model of HCC, sorafenib treatment inhibited tumor growth and augmented antitumor immune responses by inducing tumor-specific T-effector functions and attenuating the expression of PD-1. CD8+ T-cells and Tregs via apoptosis [59].

PD-1 inhibiting antibodies nivolumab and pembrolizumab have been demonstrated to prolong overall survival in randomized trials of patients with metastatic melanoma and advanced non-small cell lung cancer. In addition, pembrolizumab has shown promising results among patients with metastatic HCC who have failed sorafenib [60]. There is a marked heterogeneity, however, in the therapeutic response, which may be due to different subsets of PD-1 expressing T-cells, and hence serve as a predictor of therapeutic response [61]. Furthermore, it has been hypothesized that intratumoral hypoxia induced by sorafenib treatment may confer resistance to anti-PD-1/PD-L1 therapies. Intratumoral hypoxia may promote immunosuppression via upregulation of PD-L1 and stromal cell-derived 1-alpha [62,63]. In a HCC mouse model, the antitumor activity of anti-PD-1 antibody as an adjunct to sorafenib treatment was evident only when combined with AMD3100, a stromal cell-derived 1-alpha-receptor inhibitor. These data emphasize that combining anti-PD-1 immunotherapy with concomitant targeting of the hypoxic microenvironment after sorafenib treatment may be important [62].

The interim analysis of a phase I/II CA209-040 trial (ClinicalTrials.gov Identifier: NCT01658878) were reported in 2015, which demonstrated the safety and efficacy of nivolumab among patients with advanced HCC [64]. The incidence of complete response, partial response and stable disease was 5%, 18%, and 46%, respectively [64]. Of note, responders had a durable response with 72% alive at 6 months. Several other clinical trials targeting PD-1/PD-L1 for the treatment of advanced HCC are currently ongoing (Table 2). Specifically, a phase III study of nivolumab versus sorafenib as first-line treatment in patients with advanced HCC (ClinicalTrials.gov Identifier: NCT02576509), as well as a phase III study of anti-PD-1 antibody pembrolizumab versus best supportive care for second-line advanced HCC who progressed on sorafenib therapy (ClinicalTrials.gov Identifier: NCT02702401) are underway.

8. Conclusions

PD-1 and its ligands (PD-L1 and L-2) play a critical role in the inhibition of the immune system by inhibiting T-effector cells and inducing immune tolerance. The inhibitory effect of PD-1 is achieved through a dual mechanism of inducing apoptosis in antigen specific T-cells, as well as decreasing apoptosis of Tregs. These effects shape the overall immunity against chronic liver infections and liver malignancies. Restoration of cytotoxic T-cells by blocking the PD-1/PDL-1 pathway may be a useful strategy for liver tumor immunotherapy. The systemic administration of PD-L1/PD-1 blocking antibodies may, however, affect peripheral tolerance and cause side effects. As such, further investigation of PD-1 pathway blockade as a principal or adjuvant therapy for liver malignancies will be necessary.

References


