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Immunotherapy of hepatocellular carcinoma: strategies for combinatorial intervention

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Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer, leading to 74.6 thousand deaths annually. The prognosis of HCC over the last few decades has remained unsatisfactory, and over half of patients with early-stage HCC develop recurrence by the time of follow-up. Immunotherapeutic intervention has emerged as a novel, effective treatment to delay the progression of aggressive tumors and suppress tumor recurrence and metastasis. However, few clinical immunotherapy trials have been conducted in HCC patients, and there is an unmet need for novel therapeutic strategies. The combination of conventional treatments with specific immunotherapeutic approaches may dramatically improve the efficacy of HCC treatment and the clinical outcome of HCC patients. In this review, we briefly summarize immunotherapy strategies and discuss new advances in combined immunotherapeutic approaches for the treatment of patients with liver cancer.

hepatocellular carcinoma, immunotherapy, immune checkpoint inhibitor, strategy, combination therapy

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer deaths worldwide (Forner et al., 2018). As a curative treatment for liver cancer, liver resection provides a favorable prognosis for patients with early-stage HCC (Ueno et al., 2015), but over 60% of patients develop recurrence 5 years after surgery (Shim et al., 2015). In patients with advanced-stage HCC, current therapeutic options are limited at the initial diagnosis, since even treatment with sorafenib delivers a survival benefit of only 3 months.

Since the approval of cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) inhibitors for use in the treatment of melanoma, immunotherapy is rapidly gaining broad attention in clinical practice as a potential alternative therapeutic option for malignant tumors. HCC is an inflammation-driven disease with underlying chronic liver inflammation and cirrhosis (Llovet et al., 2016). One quarter of HCC cases express inflammatory response markers, including an adaptive T cell response and an exhausted immune response, but these tumors also have fewer chromosomal aberrations, which suggests that these immune characteristics may lead to better immune interventions in combination with conventional treatments for this deadly disease. In the present review, we summarize the main strategy of immunotherapies and discuss recent advances in synergistic combination strategies in hepatocellular carcinoma.

The current stage of immunotherapy

Immunotherapeutic approaches are primarily classified into

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five categories: adoptive cell therapies; cytokines; vaccines; immune checkpoint inhibitors; and oncolytic viruses. Table 1 summarizes the subsets, targets and clinical efficacy of relevant immunotherapies.

Strategies of combinatorial therapy

Combination of immunotherapies

The combination of PD-1 and CTLA-4 inhibitors is widely accepted in clinical trials and management. Evidence has demonstrated that blockade with both PD-1 and CTLA-4 inhibitors can provide longer survival and higher response rates for certain cancers, such as melanoma (Long et al., 2018), metastatic esophagogastric cancer (Janjigian et al., 2018) and advanced renal-cell carcinoma (Motzer et al., 2018). Blockade of the CTLA-4 antibody on Tregs and effector T cells contributed to the antitumor activity of this combined therapy. Also, the level of intratumoral Tregs is decreased by an antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. A phase II clinical trial with tremelimumab enrolled 21 patients with advanced HCC, and the partial response and disease control rates were 17.6% and 76.4%, respectively (Sangro et al., 2013). PD-1 is expressed on B cells, T cells, natural killer (NK) cells and dendritic cells (DCs). PD-1 mAbs block the receptor binding of PD-L1 and PD-L2 to activate immune cells. Research has revealed that the PD-1 inhibitor nivolumab activated sustained tumor-specific immune responses with manageable adverse effects. The rationale behind the combined PD-1 and CTLA-4 treatment is the stimulation T-cell activation to enhance tumor eradication. Several current phase I/II clinical trials are evaluating treatment combinations, such as durvalumab and tremelimumab (NCT02519348), nivolumab and ipilimumab (NCT01658878 and NCT03222076), in patients with hepatocellular carcinoma.

Immunotherapy and locoregional therapy (LRT)

LRT, including radiofrequency ablation (RFA), radiation and transarterial chemoembolization (TACE), plays a key role in the management of HCC patients. RFA is a commonly applied procedure and is considered a first-line treatment in early-stage HCC patients, while TACE is a palliative option to eliminate the tumor vascular supply. All types of LRT induce the release of neoantigens, activate the peripheral immune response and enhance the clinical efficacy of immune interventions.

Duffy and colleagues (Duffy et al., 2017) reported that tremelimumab treatment with RFA produced partial response in 26% of patients and 6- and 12-month probabilities of tumor progression-free survival (PFS) of 57.1% and 33.1%, respectively. In a retrospective study, 62 early stage HCC patients were enrolled and divided into a radical RFA group (n=32) and an RFA/cellular immunotherapy (CIT) group (n=30). NK cells, γδT cells and cytokine-induced killer (CIK) cells were measured in the RFA/CIT group. The PFS of the RFA/CIT group was significantly better than that of the RFA group, and no toxic effects were observed in the CIT group (Cui et al., 2014). In addition, the efficacy and safety of DC-based immunotherapy was investigated in a randomized phase II study. Interestingly, DC-based immunotherapy reduced the risk of recurrence in the non-RFA group but unexpectedly increased the risk of recurrence in the RFA group (Lee et al., 2017). These clinical data suggest that DC immunotherapy may not be compatible with RFA treatment in HCC patients.

Immunotherapy and molecular-targeted therapy

A number of emerging therapeutic biomarkers are promising for liver cancer treatment, including the TERT promoter (Totoki et al., 2014) and CTNNB1 (Schulze et al., 2015), FGFR4 (Hagel et al., 2015), MET (Abou-Alfa et al., 2018) and VEGFA (Horwitz et al., 2014) genes. Various clinical trials associated with novel targets, such as the TERT promoter (NCT00444782) and CTNNB1 (NCT02069145) and FGF19 (NCT02508467) genes, are ongoing. The combination of immune- and molecular-targeted strategies is needed and may offer hope for HCC patients.

In recent years, this combinatorial treatment for liver cancer mainly focused on immune-checkpoint inhibitors and multikinase inhibitors, including sorafenib, regorafenib and lenvatinib. However, the combination of PD-1 mAbs and sorafenib may not function synergistically. Chen et al. (Chen et al., 2015) revealed that the PD-1 inhibitor produced no additional antitumor response in combination with sorafenib in an orthotopic HCC mouse model. Increased hypoxia after sorafenib treatment resulted in a suppressive immune microenvironment by increasing the density of intratumoral Tregs and M2 macrophages; but, the administration of a CXCR4 inhibitor prevented the polarization toward an immunosuppressive status after sorafenib and PD-1 inhibitor treatment. An understanding of the intricate mechanisms in the tumor microenvironment may facilitate the improved clinical efficacy and safety of combination strategies.

Immunotherapy and chemotherapy

The rationale of chemotherapeutic agents is to destroy highly replicating neoplastic cells across doses. In mouse models, cisplatin enhanced the efficacy of NK cell immunotherapy by altering androgen receptor-UL16-binding protein 2 signals (Shi et al., 2016). However, HCC is highly refractory to traditional systemic chemotherapy in clinical practice due to its heterogeneity and different etiologies, and few patients exhibit long-term survival with chemotherapy. Our previous study (Qin et al., 2013) included 371 advanced or metastatic HCC patients, and no significant difference in median

Table 1 Major immunotherapeutic strategies for HCC

Approaches	Subsets	Targets, applications or therapies	Clinical outcomes
Adoptive cell therapies	CIKs	A broad spectrum of tumor-associated antigens (TAAs)	Provided better recurrence-free survival (RFS) in patients with adjuvant interleukin (IL)-2-induced CIKs postoperatively (Lee et al., 2015)
	TILs	Multiple tumor antigens	Improved recurrence-free survival and recurrence frequency (Takayama et al., 2000)
	NK cells	Specific ligands on tumor cells, such as MHC class I related chain A and B	Showed good clinical effectiveness (Alnaggar et al., 2018)
	CAR T	Targets TAAs and kills tumor cells in an MHC-independent manner	Antigen recognition is HLA-dependent; showed promising results in melanoma and synovial carcinoma
Cytokines	IFN-α	IFN-α-2b	Disappointing results concerning its antitumor efficacy (Chen et al., 2012; Llovet et al., 2000); did not prevent HCC recurrence (Mazzaferro et al., 2006)
	IL-12	Direct injection of an adenovirus containing IL-12 coding sequence; transfection of infected dendritic cells with IL-12 virus	No antitumor efficacy (Mazzolini et al., 2005)
	TGF-beta receptor inhibitor	Galunisertib	Improved survival with an OS of 36 weeks in a phase II trial
		Activin receptor-like kinase-1 (ALK1) antibody, PF-03446962	50% of patients achieved stable disease; requires further investigation (Simonelli et al., 2016)
Vaccines	Cell vaccines	Autologous or allogenic HCC cells or lysates	Uncertain due to its weak immunogenicity
	Antigen peptide vaccines	AFP, GPC3, NY-ESO-1, hTERT and MAGE-A	Lowered recurrence in patients who underwent surgery and GPC3-derived vaccination (Sawada et al., 2016)
	DC vaccines	All TAAs, irradiated tumor cells and CEA	
Immune checkpoint inhibitors	CTLA-4	Tremelimumab	26.3% of patients achieved partial response (Duffy et al., 2017)
	PD-1	Nivolumab, Pembrolizumab	Activated sustained tumor-specific immune responses with manageable AEs (El-Khoueiry et al., 2017)
	PD-L1	Durvalumab	No clinical trials involving the use of PD-L1 inhibitors for the treatment of HCC have been conducted
Oncolytic viruses		CVV, JX-594, GLV-1h68	JX-594 treatment led to an intrahepatic response rate of 62% and an intrahepatic disease control rate of 50% (Heo et al., 2013)

overall survival was observed between FOLFOX 4 (6.40 months) and doxorubicin (4.97 months) groups. Further, Murawski and colleagues (Murawski et al., 2016) confirmed that the intensification of platinum agents did not improve survival in pediatric patients with hepatocellular carcinoma.

Novel approaches are needed for the systematic treatment of liver cancer. Palbociclib is a new cell-cycle target in cancer that has been found to inhibit cyclin D-dependent kinase (CDK) 4 and 6 (O'Leary et al., 2016; Sherr, 2016) and improve survival in advanced breast cancer (Burki, 2017). Gianni et al. revealed that 97% of patients who underwent palbociclib and targeted therapy had clinically objective responses, and 27% of patients had a pathologically complete response in breast and axillary nodes (Gianni et al., 2018). Further, CDK20 inhibition was reported to diminish the immunosuppressive function of $CD11b^{+}CD33^{+}HLA\text{-}DR^{-}$ myeloid-derived suppressor cells and enhance immune-checkpoint blockade efficacy to eradicate HCC (Zhou et al., 2018). In a preclinical model of HCC, palbociclib showed encouraging results by impairing tumor growth in vivo and in vitro and significantly prolonging survival. Nearly 70% of patients treated with palbociclib benefited from its RBI-proficient signature (Bollard et al., 2017). The combination of novel chemotherapeutic agents and immunotherapy may facilitate treatment susceptibility in liver tumors, especially for those that are not responsive to traditional strategies.

Immunotherapy and surgical resection

Liver resection is a curative treatment for HCC patients, but studies that have focused on immunotherapy and liver resection are limited. In a randomized trial, Takayama (Takayama et al., 2000) reported that adoptive immunotherapy could lower postoperative recurrence rates. In this trial, autologous lymphocytes were activated with IL-2 and CD3 antibodies in vitro and these cells were reinfused 5 times during the first 6 months. The median time to first recurrence was 2.8 years and 1.6 years in the immunotherapy and control groups, respectively. However, no significant difference in overall survival (OS) was observed in either group. Recently, Lee and colleagues (Lee et al., 2015) performed a multicenter, randomized phase III clinical trial to investigate the efficacy of autologous cytokine-induced killer cells (CIKs) in liver cancer; all the patients had previously received liver resection, RFA or percutaneous ethanol injection (PEI). Compared with the control group (30.0 months), the

Table 2 Predictive biomarker strategies for immunotherapy

Biomarker	Details of approach	Targeted cells or tissues	Improved clinical outcomes
PD-L1	Immunohistochemistry	Immune cells and tumor cells	Low PD-L1 expression on tumor cells (Calderaro et al., 2016)
		Macrophages	High PD-L1 expression on macrophages (Liu et al., 2018)
		Hepatoma cells, predominantly	Low PD-L1 expression in CIK group (Chen et al., 2016)
TILs	Immunohistochemistry	$CD8^+$ cells	Increased the density of $CD8^+$ TILs (Sideras et al., 2017)
		Cytotoxic T cells	Cytotoxic T cells at tumor margins associated with response, higher clonal expansion of TCR in responders (Tumeh et al., 2014)
Mutational and neoantigen burden	Whole or targeted exome sequencing	Tumor tissues	High mutational count (Brown et al., 2014; Rizvi et al., 2015; Van Allen et al., 2015), high neoantigen count (Inada et al., 2019; McGranahan et al., 2016)
Gene expression	Multiple gene expression signature	Tumor tissues	Interferon γ or T-cell infiltration

median recurrence-free survival (RFS) in the immunotherapy group was 14.0 months longer. The OS was longer in the immunotherapy group than in the control group. This result was confirmed in a meta-analysis of CIKs (Wang et al., 2016). Six randomized, controlled trials with 844 HCC patients were included in the meta-analysis, and the final result indicated that CIK treatment can be safely used to improve the prognosis for hepatocellular carcinoma patients after radical resection.

Predictive biomarkers for immunotherapy

Scientific advances have changed the landscape of cancer immunotherapy by improving the understanding of tumor biology and the immune network, progressing sequencing technologies and advancing the clinical applications of gene therapy. Exploring the precise genetic and immunological determinants associated with therapeutic responses may facilitate individualized cancer management with targeted or immunological therapies. Thus, identifying predictive biomarkers is of the utmost importance to promote survival benefits in tumor patients. Currently, biomarkers mainly focus on aspects of the inflamed T-cell phenotype and the tumor foreignness that is associated with clinical outcomes for CTLA-4, PD-1 or PD-L1 inhibitors (Calderaro et al., 2016; Chen et al., 2016; Liu et al., 2018). Genetic markers predicting responses to various immune therapies, such as defective DNA mismatch-repair (Le et al., 2017) and IFIT3 (Yang et al., 2017), might be helpful in the clinical setting (Table 2).

Conclusion

Combined therapeutic approaches using immune intervention are a promising strategy for the treatment of HCC patients, especially for those in advanced stages. However, several concerns need to be addressed to improve the clinical outcome of tumor immunotherapy and expand the indications in the treatment of HCCs: (i) identification of patients suitable for immunotherapy by exploring the novel predicting biomarkers; (ii) improvements in the anti-tumor effects with another immunotherapy or conventional therapies; (iii) determination of the optimal combinatorial strategies for HCC on the basis of further clinical researches; (iv) development of novel immunotherapies; and (v) establishment of the selection criteria for immunotherapy. The evaluation of tumor response may be difficult for its delayed effect. Thus, the criteria evaluating the subsequent effect of immunotherapy precisely may be of great clinical significance for HCC patients. Future clinical trials or researches should be designed to study these elements, which might be performed by the incorporation of pre- and post-treatment biopsies based on scientific rationale. When these challenges have been overcome, the cancer immunotherapy will change the future landscape of HCC treatment.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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