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Immunotherapy: a new era for hepatocellular carcinoma

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Abstract

Cancer is a major disease threatening human health. The overall prognosis for hepatocellular carcinoma (HCC) patients is poor, with a dismal 5-year survival rate of approximately 5%-30%. The dysfunction of immune system plays a pivotal role in the development of cancer, which has attracted attention of several researchers. Recent advances in immunotherapy have led to various inspired achievements and refreshed our concepts about cancer treatments. In this article, several types of immune-based therapies for treating HCC are reviewed. Their underlying mechanisms, preclinical and clinical study results, potential prospects, and deficiencies are discussed, and an outline for future research directions is proposed.

Keywords: Hepatocellular carcinoma, immunotherapy, cancer treatments

INTRODUCTION

Cancer is one of the primary diseases that threaten human health. Nearly 14.1 million new cases of cancer and 8.2 million cancer-related deaths worldwide were estimated in 2012. Moreover, 782,000 new cases of liver cancer have been recorded, with nearly half of these cases reported in China alone[1]. Liver cancer is the second most common cause of cancer deaths among adult men worldwide. Nearly 746,000 deaths (9.1% of the total) were caused by liver cancer in 2012. Hepatocellular carcinoma (HCC) is the most primary,

common, malignant liver cancer. The overall prognosis for HCC patients is poor, with a dismal 5-year survival rate of approximately 5%-30%[1,2].

The most common progression of liver cancer is from chronic inflammation to cirrhosis and eventually developing to HCC through a prolonged period leading to multiple function disorders. Immunosuppression may be one of the most important reasons. T cell dysfunction, also known as T cell exhaustion, occurs in chronic infections and cancers. Various cell populations, including infiltrating immune cells and tumor cells, stroma cells with related cytokines and metabolites, cause T cell dysfunction in the tumor microenvironments. Exhausted T cells lack robust effector functions and express multiple inhibitor receptors that reduce efficient immunological surveillance of tumor[3,4]. Tumor recurrence and relapse-free survival (RFS) are correlated to CD3+, CD8+ immune cells or tumor infiltrating lymphocytes (TILs), as well as the inhibitory receptors such as programmed cell death protein 1 (PD-1) and its ligand[5]. The potential immunosuppressive mechanism involves the hepatoma-intrinsic cell cycle-related kinase (CCRK) signaling stimulated by the expansion of the polymorphonuclear (PMN) myeloid-derived suppressor cells (MDSCs), which have been correlated to potent T cell suppression and poor prognosis of patients[6,7]. Expression of ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2) on the surface of cancer cells is induced by hypoxia, which elevates extracellular 5'-AMP and prevents the differentiation of MDSCs, consequently, contributing to the maintenance of MDSCs[8].

Recently, cancer immunotherapy has emerged from being an adjacent to a frontline therapy and has demonstrated positive outcomes involving various cancers. Antagonistic antibodies for the PD-1 and cytotoxic T cell lymphocyte antigen-4 (CTLA-4) pathways have been approved by the Food and Drug Administration (FDA) for use in a growing number of cancers, including Hodgkin's lymphoma (HL), melanoma, bladder, non-small-cell lung and kidney cancers[9,10]. Tumor tissue deep sequencing has advanced the neoantigen-based vaccines[11,12] and neoantigen-specific T cells[13-16] to clinical trials and resulted in discovering significant antitumor effects that will make individualized immunotherapy become a reality. In 2017, 2 kinds of chimeric antigen receptor (CAR) T cells that target CD19 have received FDA approval for treatment of diffuse large B-cell lymphoma (DLBCL) and acute lymphoblastic leukemia (ALL), respectively[17-19]. Here, we provide an overview of current preclinical and clinical immunotherapeutic approaches for HCC.

IMMUNOTHERAPY APPROACHES

Cancer vaccine

Promoting tumor specific immune responses, especially the cytotoxic CD8+ T cells is the main goal of cancer vaccines. In colorectal cancer (CRC), breast cancer and ovarian cancer, the reduced frequency of tumoral cytotoxic CD8+ T cells is correlated with poor disease prognosis[20-23]. On the other hand, it is a positive prognostic factor in that TILs are present in tumor deposits. The investigation of vaccines that target specific mutated antigens is being encouraged due to the technological developments in the recent few years. Several kinds of cancer vaccines are being tested, for instance proteins, peptides, tumor cells, antigen presenting cells (APC), and viral vectors.

Vaccination with antigens

The first step toward DC vaccine production is loading tumor antigens on the immature dendritic cells (DCs). Tumor antigen candidates could be mutated genes, neoantigens, viral genes, tissue-specific genes, whole proteins, deoxycholate citrate sugar (DCA) constructs and tumor lysates of autologous or allogeneic tumor cells or tumor cell lines, which belong to either tumor-associated antigens (TAAs) or tumor-specific antigens.

Alpha-fetoprotein (AFP) is a fetal serum protein produced in the liver and is normally synthesized only during fetal development until shortly after birth, while it is produced again in instances of HCC. Specific

cytotoxic T lymphocytes (CTLs) targeting against this antigen have been shown to exist in the T cell repertoire, without being peripherally or centrally deleted, which suggests AFP is a promising target antigen for HCC immunotherapy[24,25]. Human T cell repertoire could effectively respond to the AFP self-antigen in the context of major histocompatibility complex (MHC) class I or after the administration of AFP peptide-pulsed DC[26,27]. Previous studies using DCs or T cells pulsed with AFP-derived peptides suggest that AFP-derived peptides are suitable epitopes as immunotherapy targets. However, because of the self-nature of AFP, the vaccine-activated immune responses were weak. Thus, not surprisingly, the clinical results were not satisfactory[27,28] except that a recent phase 1 clinical trial in HLA-A24 patients showed that immunization with AFP-derived peptides resulted in immune responses in 33% (5 of 15) of patients, of whom one patient had complete response[29]. To enhance the AFP-specific immune responses, investigators mutated the AFP epitope to create epitope-optimized vaccines. They recently found that epitope-optimization of AFP antigen together with genetic immunization can activate potent AFP-specific CD8 responses[30]. The activated CD8+ T cells in mice could not only cross-recognize short synthetic wild-type AFP peptides, but also identify and kill the tumor cells expressing wild-type AFP, which successfully prevents the immunized mice from developing carcinogen-induced autochthonous HCC. Further studies show that the antitumor effects of vaccine-activated AFP-specific CD8 T cells are correlated to optimal T cell receptor (TCR) signaling strength and induction of stem-like memory T cells[31,32].

Conversely, cancer vaccine development benefits from deep sequencing and rapid identification of neoepitopes in the tumor lesions[33], a modern technology has emerged for designing a personalized immunotherapy approach by using neoantigens -mutated antigens generated in the tumor mass that are unique to each patient's cancer to elevate the immune function and kill cancer cells. The identification of personalized somatic mutations can be conducted by whole-exome sequencing and matching DNA from normal cell with tumor cell from each patient. Mutated peptides are then synthesized to create a new vaccine that has a high likelihood to bind to the autologous human leukocyte antigen (HLA)-A or HLA-B proteins. In a phase I clinical study for melanoma with neoantigen-based vaccines, 15 (16%) and 58 (60%) of the 97 unique neoantigens could be targeted by vaccine-induced polyfunctional CD8+ and CD4+ T cells, respectively. No recurrence of tumor was noticed in 4 of the 6 vaccinated patients for up to 25 months after vaccination.

Vaccination with APC

Antigen presenting cell, including dendritic cells, activated B cells, and peripheral blood mononuclear cells, have been widely investigated as candidates for tumor vaccine. In the innate immune system, the most efficient APCs are the DCs[34,35]. They are well-known to be the most potent APCs for inducing antigen-specific T cell responses. They acquire and present tumor antigens to T lymphocytes, promote the generation of CTLs and helper T cells[36], decrease the proportion of CD4+CD25+ regulatory T cells[37] and induce anti-tumor immune response[38]. After the first DC-based vaccine for the treatment for prostate cancer, the study of DCs is continuously growing internationally. DC-based therapies are increasingly investigated and used to treat many kinds of patients with cancer or other diseases. In terms of manufacturing the DC vaccines, it is important to select proper tumor antigens and choose the appropriate method for loading the tumor antigens onto the DCs. Tumor antigen-pulsed DC vaccines can effectively develop mature DCs (mDCs) and enhance T cell stimulation to generate potent CTLs.

There are 3 generations of DC vaccines according to the development of different subsets. First-generation DC vaccines are not fully matured, consisting of patient-derived natural DCs or monocyte-derived DCs (mo-DCs). Antigens, such as tumor cell lysates or recombined/synthetic antigenic peptides, are loaded onto the DCs *ex vivo* and then reinjected in the patients. The first-generation DC vaccines provided satisfactory outcomes in terms of safety and feasibility but not of expected clinical efficacy[39-41]. The second-generation DC vaccines consisted of mo-DCs matured via maturation cocktails. Such vaccines are widely used in the clinics because of its minimal immunogenic side effects and better clinical responses[42]. Nowadays, the clinical progress on DC vaccines has reached a new era: next-generation DC vaccines. Many defined DC subsets

(including patient-derived and mo-DCs) confer the next-generation DC vaccines superior functionalities for presenting MHC-I/II antigen and eliciting CTL responses[43]. Pulsing DCs with CD44 and epithelial cell adhesion molecule (EpCAM) peptides can activate cancer stem-like cells (CSCs) peptide-specific immune responses leading to better clinical outcomes when combined with standard chemotherapy for advanced carcinomas[44]. Cytoplasmic transduction peptide (CTP), a novel antigen delivery tool, can transduce tumor antigen such as the forkhead box protein M1 (FoxM1) into the cytosol of DCs[45].

Vaccination approaches

Many studies have focused on pre-conditioning the DC-based vaccine sites and have already reported some interesting discoveries. The lymph node homing and immune function of tumor antigen-specific DCs can be significantly improved by pre-conditioning the vaccine site with a potent recall antigen, such as tetanus/diphtheria (Td) toxoid. A significant increase in both PFS and overall survival (OS) in Td-treated patients compared with DC-treated patients has been approved for clinical trials[46]. Furthermore, RNA-lipoplexes (RNA-LPX) encoding endogenous self-antigens or mutant neo-antigens or viral can enable precise and effective targeting of DCs and perform effectively *in vivo*, as well as induce strong effector and memory T cell responses. This could result in a universally applicable vaccine type for DC based cancer immunotherapy[47]. Exosomes derived from AFP-expressing DCs (DEX_{AFP}), another type of vaccine for cancer immunotherapy elicits strong antigen-specific immune responses and restructures the microenvironment in tumor[48]. DCs can also be loaded via RNA transfection[49] or recombinant viral transduction[50].

Immune checkpoints-specific antibodies

The interactions between an APC and a T cell through the TCR-antigen/MHC complex simultaneously trigger both co-stimulatory and co-inhibitory signals. The balance between these signals determines the overall activation and function of T cells. Several co-inhibitory molecules (PD-1, CTLA-4, BTLA-4, LAG-3, TIM-3 and CD160) expressed on the surface of T cells are the targets of antibodies[51-55]. Checkpoint blocking antibodies have been approved by the FDA since 2014 for patients with lung cancer, melanoma, and other tumors. For HCC, CTLA-4 and PD-1 antibodies have been intensely investigated and are both advancing to the clinical trial stage.

CTLA-4

Blocking CTLA-4 induces a strong antitumor immune response[56], and research on CTLA-4 is ongoing[57,58]. CTLA-4 blockers were mainly ipilimumab and tremelimumab. In 2011, FDA approved ipilimumab for the treatment of melanoma. However, for the CTLA-4 molecular targeted therapy, only tremelimumab is currently undergoing clinical trials related to liver cancer. In a phase II clinical trial of tremelimumab[59], median OS was 8.2 months and median TTP was 6.48 months among all 21 patients enrolled. Among the 17 patients continuously treated with tremelimumab, no complete remission (CR) was observed, while 3 patients (17.6%) had confirmed partial remission (PR) that was maintained up to 3.6, 9.2 and 15.8 months, respectively. Overall, a good safety profile was recorded and no treatment-related death occurred. The feasibility and safety of tremelimumab combined with ablation (chemoablation or radiofrequency ablation) in patients with advanced HCC was assessed in another clinical trial[60]. Among the 19 patients evaluated, 5 patients (26%) achieved confirmed PR. The median OS was 12.3 months and median TTP was 7.4 months with a median potential follow-up of 18.8 months for the total study population (n = 28). Tremelimumab was well tolerated across the different dose cohorts and no dose-limiting toxicities (DLT) was encountered. Recently, ipilimumab, another drug combined with the fully humanized anti-CTLA-4 IgG1 antibody, has been investigated in several clinical trials. These results have not been published.

PD-1/PD-L1

PD-1 is expressed on T cells binding with its ligand (PD-L1, PD-L2)[61,62]. PD-L1 is expressed on APC[63] and negatively regulates downstream signals of T cell receptor stimulation to reduce T cell activation and cytokine

production, while decreasing tumor-killing ability[64,65]. PD-1, lymphocyte-activation gene-3 (LAG3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3) and CTLA4 are expressed on CD4+ and CD8+ T cells, as well as B cells and natural killer (NK) cells[66]. The expression of TIM-3, PD-1, CTLA4, and LAG3 was significantly higher in CD4+/CD8+ T cells and TAA-specific CD8+ TILs in the HCC tissue than in the control tissue or blood. Blocking these immune checkpoints may increase *ex vivo* proliferation and effector cytokine production of tumor-infiltrating T cells[67]. Thus, the anti-tumor immune response of immune cells can be enhanced, and tumor growth controlled[64,68]. Nivolumab, a fully human IgG4 monoclonal antibody PD-1 inhibitor was investigated in a multiple ascending-dose, phase I/II study in HCC patients. In 39 patients whose response could be evaluated, 2 CR (5%), and 7 PR (18%) cases were reported. Response duration was 14-17 or more months for CR, less than 1-8 or more months for PR, 1.5-17 or more months for stable disease, and an OS of 72% at 6 months. The toxicity profile has been well managed[69]. Subsequently, another randomized, multi-center clinical trial comparing the efficacy with nivolumab *vs*. sorafenib is ongoing (NCT02576509). Besides, the overall expression of PD-L1 on tumor cells is negatively correlated with tumor recurrence and survival in HCC patients. It can be used as an independent prognostic factor for the disease-free survival of patients with liver cancer[70,71]. Currently, plenty of early clinical trials of PD-1/PD-L1 blockers alone or in combination with CTLA-4 blockers for liver cancer are ongoing. At present, the FDA has already approved 5 PD-1/PD-L1 checkpoint blocking antibodies for non-HCC tumors, 2 are PD-1 antibodies: nivolumab, pembrolizumab; 3 are PD-L1 antibodies: durvalumab, atezolizumab, and avelumab. Furthermore, nivolumab has been approved by FDA for HCC patients who received sorafenib treatment in the USA in September 2017. The clinical efficacy of each drug in controlling HCC will be worth anticipating.

Adoptive cell therapy

Adoptive cell therapies (ACTs) that expand certain cells *ex vivo* and then infuse them back to patients have in recent years gained attention for the clinical treatment of tumors. These modified cells are able to transfer to the site of tumor and mediate its destruction[72]. Modified strategies are mainly focused on T cells especially the CD8+ T cells that perform specific tumor killing function[15].

CIK/DC-CIK immunotherapy

CIK/DC-CIK is one of the ACTs that can expand autogenous T lymphocytes *ex vivo* and are stimulated by many kinds of cytokines co-cultured with DC pulsed by tumor antigens alternatively[73]. After culturing, CIK cells would comprise of CD3+CD56+ cells, CD3+CD56- cytotoxic T cells, and CD3-CD56+ NK cells. These heterogeneous cells are characterized by dual functions, acting both as NK-like and CD8+ specific effector T cells[74]. At the same time, CD8+ specific effector T cells can specifically be activated by DC loaded with tumor antigens. A multicenter, randomized, open-label, phase III trial on the efficacy and safety of adjuvant immunotherapy with activated CIK cells showed that the median time of recurrence-free survival (RFS) was 44 months in the immunotherapy group and 30 months in the control group of patients with HCC when subjected to curative treatment[75]. Given that the efficacy of immunotherapy is primary influenced by the complex immune microenvironment in HCC patients, immune factors should be considered for and may represent additional prognostic parameters for predicting survival benefits of immunotherapy. In addition, adoptive CD8+ T cells cannot be replicated *in vivo* after infusion, though it can be expanded abundantly *ex vivo*. Therefore, CIK/DC-CIK need to be transfused repeatedly to achieve better clinical efficacy. In a retrospective study of 448 HCC patients that received complete hepatectomy combined with/without CIK cell immunotherapy, the prognosis was significantly improved in the CIK treatment group compared with the surgery only group. Higher PD-L1 expression predicts better OS and RFS, especially in the subgroup with high hepatitis B viral load[76]. However, another clinical trial reported no significant differences in DFS and OS between the patients who received CIK (n = 100) and who did not (n = 100) after curative hepatectomy[77]. The clinical efficacy of CIK/DC-CIK treatment needs to be further demonstrated.

Genetically engineered T cells

As a pivotal role in killing tumor cell, the function of T cells has always been the focus of investigation. With the development of modern genetic techniques, T cells can be genetically engineered for enhanced anticancer immune functions. These engineered T cell therapy has been first applied in hematological malignancy[78,79] and then gradually introduced to treat solid tumors such as glioblastoma[80], prostate cancer[81] and sarcoma[82]. Recent studies on modified T cells expressing engineered TCRs and CARs show encouraging results to advance from basic to clinical research.

TCR engineered T cells

Endogenous TCRs recognize the peptide segments submitted by MHC-I and MHC-II on the cell surface with a heterodimer consisting α- and β-chains. Each TCR is a heterodimer that determines the TCR antigen-specificity. TCR-T was genetically modified with TCR chains for targeting specific antigens expressed on tumor cells to cure specific diseases. As the peptides were processed and submitted by MHC, they present various antigens as an expanded pool of potential targets. For this reason, TCR-T can target moreantigens in comparison to CAR-T[83]. It was the first successful application of ACT when 17 patients with metastatic melanoma were treated using autologous T cells transduced with TCR recognizing the MART-1 melanoma-melanocyte differentiation antigen[84]. Although objective cancer regressions were observed in mice and expanded clinical trials, severe "on-target, off-tumor" toxicity occurred in the skin, eyes, and ears of patients because of the expression of antigenic targets in these organs[85,86]. AFP and GPC3 are commonly expressed in the HCC. These two specific antigens are good targets for engineered T cell therapy. Peptide $GPC3_{367}$ is a predominant peptide identified on HLA-A2 positive hepatoma cells. CD8(+) T cells that express $GPC3_{367}$-specific T cell receptor can recognize and kill GPC3-positive hepatoma cells and reduce growth of HCC xenograft tumors in mice[87]. In a recent study, novel AFP-specific murine TCR genes have been identified that can redirect human T cells to specifically recognize and kill HCC tumor cells[32]. AFP-specific murine TCR genes were identified in another study. These TCR-T cells specifically recognize HLA-A*02:01$^+$/AFP$^+$ HCC tumor cells and produce effector cytokines to kill them *in vitro*. Adoptive transfer of TCR-T cells prevent and regress HepG2 tumor outgrowth in NSG mice, irrespective of CD4 or CD8 TCR-T cells. Though tumor developed in one of the TCR-T-treated mice, it was eradicated 3 weeks after transfer[32]. HBV infection is one of the most common causes of HCC tumorigenesis. In one case report, a patient seems to have developed HCC relapse 10 years after liver transplantation for HBV+ HCC. At the time of HCC relapse, HBsAg (but not HBV DNA) was detected in the blood analysis, while HBsAg, HBcAg and HBV DNA were negative in liver biopsies for the transplanted liver. Subsequently, HCC autologous T cells genetically modified to express an HBsAg specific T cell receptor were transferred to this patient. The results show reduced levels of HBsAg without exacerbation of liver inflammation or other toxicity, while clinical efficacy could not be established. This leads to a novel strategy of personalized immunotherapy targeting specific peptides in the treatment of HBV associated HCC[88].

TCR-T therapy has got into clinical trial of multiple myeloma (MM), metastatic melanoma and esophageal cancer, while the safety reports differ from each other. In a phase I trial of MAGE-A4 T cell receptor gene-transduced lymphocytes in patients with recurrent esophageal cancer, none of 10 patients experienced any adverse events for the first 14 days after T cell transfer[89]. However, the safety is not optimistic in other 2 trials. Seven of 20 patients with MM had SAEs after infusion of NY-ESO-1 specific TCR engineered T cells[90]. While 2 of 14 patients had serious adverse events (SAEs) of acute respiratory distress requiring intubation associated with patchy pulmonary infiltrates within 1 week of cell infusion with MART-1 T cell receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma[91]. Therefore, the toxicity may bring new challenges to the development of TCR-T therapy. Recently, 4 TCR-T therapies in HCC have started phase I/II clinical trial (NCT02686372, NCT02719782, NCT03441100, NCT03132792). The safety of these trials needs to be paid significant attention as well as clinical efficacy.

Chimeric antigen receptor engineered T cells

Unlike TCRs, CARs are formed by a combination of antibody-derived or ligand-derived domains and TCR domains. Due to engineering specific antigens, CAR-T specifically expresses a receptor to direct the T cells to target and destroy cancer cells. Therefore, CAR-T therapy represents specific recognition and lethality. Meanwhile, specificity enhancement of CAR-T cells can make them activate at very low level of target on non-malignant tissue so that prevent off-tumor toxicity. Based on the different engineered chains of CAR, CAR-T has developed from the first generation to the second and third generation[83,92]. With the development of CAR-T therapy, CAR-T cells have longer survival times, better functional properties, and less toxicity. These characteristics make CARs "living drugs" that exert both immediate and long-term therapeutic benefits. The third generation of GPC3-CAR-T cells are able to efficiently kill GPC3-positive HCC cells, while suppressing the growth of HCC xenografts. The cytotoxic effects were positively correlated to the GPC3 expression levels in the target[93,94]. A phase I clinical trial for anti-GPC3 CAR T has been sponsored in 2015 to evaluate the safety and effectiveness for patients with relapse or refractory HCC. Thirteen patients were enrolled in this trial, and the results are eagerly anticipated (NCT02395250). A different CAR-T approach towards HCC was recently developed by utilizing the antibody against HLA-A2/AFP_{158} peptide complex[95]. If successful, this approach may expand CAR-T therapy to the intracellular tumor antigen. T cells expressing ET1402L1-CAR (AFP-CAR) could selectively lyse liver cancer cells that were HLA-A*02:01^{+}/AFP^{+}. Under *in vivo* conditions, both intratumoral infection and intravenous administration of AFP-CAR T cells significantly inhibit tumor growth in mice. The robust antitumor activity was attempted in an established intraperitoneal liver cancer xenograft model. The phase I clinical trial of an ET1402L1-CAR started only in 2017 (NCT03349255). Autologous CAR-modified T cell directed CD133 (CART-133) is another therapy targeting for CD133, which has developed into a phase I trial for HCC and pancreatic carcinomas and colorectal carcinomas. The results showed 3 PR and 14 stable disease in all 23 patients. For safety, the reduction of hemoglobin, lymphocytes, and thrombocytes occurred in nearly all the patients. Lymphopenia presented in all the non-HCC patients with grade 2-4 and all HCC patients with grade 2[96]. So far, most clinical results of CAR-T have come from the treatment of hematologic diseases. The clinical trial of CAR-T for solid tumors is just beginning. However, cytokine releasing syndrome and on-target/off-tumor toxicity are still very important side effects which should be solved in either hematologic diseases or solid tumors.

Due to the complexity of the immune system post-infusion, ACT is more complex than other types of immunotherapy. For expressing the different antigens and the varied microenvironments in different patients, several biotechnology companies are turning their efforts to develop personalized approaches. This is being attempted by screening personalized tumor antigens and expanding personalized lymphocytes in the individuals. Although multiple commercial models have been proposed, the effectiveness and safety need further investigation.

SUMMARY AND FUTURE RESEARCH DIRECTION

Although the number of HCC related deaths is high, its prognosis remains poor and available treatment options are limited. Over the past decades, immunology has evolved from the basic to the clinical realm, which has contributed to many immunotherapies entering the clinics, which is encouraging and offers new treatment prospects for HCC. Strategies including immune checkpoint blockers, genetically engineered T cells (TCR-T and CAR-T) have already secured FDA approval for many types of cancer treatments. The screening and identification of HCC neoantigens have reinvigorated the relevance of immunotherapy, and precisely, pushing the personalized treatment into a reality. The progress in the field of cancer treatment is obvious, yet, tumor is still a dreadful disease with limited options to cure. Making the treatment more accurate and effective for HCC remains a huge challenge. To better understand tumor, further research of the tumorigenesis mechanism is needed. With immune suppression in tumor microenvironments, further research should likely focus on alleviating inhibition of immune suppression and restoring normal immune functions. Various immune functions also need to be further investigated including tumor antigen

presentation for APC, recognition and killing of tumor by immune cells, and function restoration of immune cells. For each of the immunotherapy strategies outlined, precision, accuracy, efficiency, thoroughness, and safety must be considered. Clinical trials and experiments should be thoroughly designed to derive real value of clinical testing. Target patients, method of administration, treatment strategy are additional factors for consideration. In addition, novel drugs and approaches are still expected to be introduced. In conclusion, there is no doubt that a new era is beginning for HCC treatment, which shines the light of hope in our quest to conquer cancer.

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Authors' contributions

Designed and drafted the manuscript: He YJ
Reviewed and modified the manuscript: Guo YB, Zhu W, He YK, Hou JL
Read and approved the final manuscript: all authors

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