

Cell-mediated immunotherapy for hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Most of the time, these tumors are diagnosed at late stages. Because no effective treatments exist for patients with advanced stage HCC, there is an urgent need for novel, effective treatments. Cancer cells originate as a consequence of abnormal expression of oncogenes or loss of tumor suppressor genes. Often, neoplastic transformation results in a hyper-mutated cellular genome, which in turn produces neo-antigens from mutated genes. These tumor-specific or tumor-associated antigens can be recognized by antigen-presenting cells and trigger T-lymphocytes to elicit anticancer immunity. Immune responses to cancers are often rendered ineffective by tumor immune-editing and immune-suppressive mechanisms. Yet, therapeutic strategies to stimulate anti-cancer immunity have had remarkable success in several solid and hematological malignancies. Among the various strategies for cancer immunotherapy, cell-mediated immunotherapy holds considerable promise to overcome anergy and systemic immune suppression. This brief review will focus on cell-mediated immunotherapy for HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy in the world^[1]. The treatment modalities for HCC included liver transplantation^[2,3], liver resection^[4], local ablation^[5], transcatheter arterial chemoembolization (TACE)^[6,7], molecular target therapy^[8,9], radiotherapy^[10], chemotherapy^[11], and so on. According to Barcelona Clinic Liver Cancer (BCLC) classification, HCC can only be cured by liver

transplantation, liver resection, and radiofrequency ablation (RFA) in the very early and early stages of the disease^[12]. Even when tumors are completely removed by liver resection and liver transplantation, or by complete ablation by RFA, tumor recurrence is still not preventable. When tumors are in the intermediate stage, they can only be controlled by TACE. When tumors are in more advanced stages, patients can only be treated by molecular target therapy or supported by best care. There are as yet no effective treatments for



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patients with advanced stage HCC, so the search for effective treatments is a crucial one.

Cancer cells occur as a consequence of enhanced or aberrant expression of oncogenes or from loss of tumor suppressor genes. Cancer cells with genetic change will express new antigens^[13]. These tumor-specific or tumor-associated antigens might be recognized by antigen-presenting cells and trigger T-lymphocytes to conduct anti-cancer immunity^[14-16]. Immunotherapy has been studied as an attractive and novel therapeutic strategy to treat cancer since a few decades ago. This brief review will focus on cell-mediated immunotherapy for HCC.

IMMUNITY IN CANCER

The immune system is the most important protection for a host in defending itself from foreign invaders and cancer development. As noted, cancer cells occur as a consequence of enhanced or aberrant expression of oncogenes or loss of tumor suppressor genes. The cancer cells with genetic change express new antigens and the new antigens may be captured and processed by dendritic cells (DCs) to trigger T cell-mediated immunity^[17,18].

Dendritic cells, the most potent and professional antigen-presenting cells, constitutively express major histocompatibility complex (MHC) class I and II and high levels of costimulatory molecules CD40, CD80, and CD86. When DCs meet antigens, they capture antigen, process it, and present the antigen to activate antigen-specific cytotoxic T-cells. Clinically, DC-based immunotherapy has been applied to treat end-stage patients with B-cell lymphoma^[19], melanoma^[20], renal cell carcinoma^[21], prostate cancer^[22] and other tumors^[23]. The results are promising^[24]. DC-based immunotherapy offers a hope of successful eradication of cancer [Figure 1].

However, DC-based immunotherapy yields only a 20% response rate in most of the clinical trials for advanced cancer diseases^[25]. These results suggest that, even though DCs are the most powerful antigen-presenting cells, the immune system of most advanced cancer patients cannot be activated or may only be activated to a limited extent by DC. It is hoped that exploration of immunosuppressive mechanisms in tumor-bearing patients will improve the success of DC-based or cell-mediated immunotherapy.

IMMUNODEFICIENCY IN CANCER PATIENTS

T-cells are the direct effector cells that attack and eradicate cancer cells. The cytotoxic ability of

Dendritic cells -- Cancer vaccine

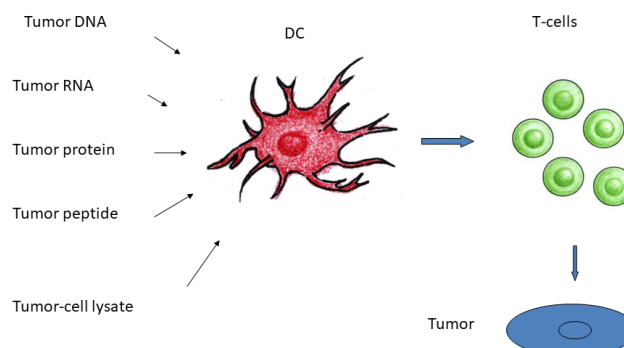


Figure 1: Dendritic cells can be pulsed by tumor DNA, RNA and proteins to activate antigen-specific T-cells. These activated T-cells can produce antigen-specific cytotoxicity to eradicate tumor cells

activated T-cells is directly related to efficacy of cancer treatment. In animal studies, tumor-infiltrating lymphocytes in tumor-bearing hosts have been proved anergic to cancer cells. Cancer cells may also induce T-cell apoptosis or regulatory T-cells, which conduct peripheral tolerance to cancer cells^[26,27]. Clinically, we have already observed that the percentage of lymphocytes decreases along with tumor growth in HCC patients^[25]. In patients with significant numerous tumor mass, the percentage of lymphocytes is always below normal range. Immediately before patients die of HCC, lymphocytes cannot even be detected. Obviously, lymphocytes are suppressed by HCC through currently unidentified mechanisms.

Regulatory T cells are immune suppressive cells^[28]. In animal models, depletion of regulatory T cells causes inflammatory colitis, and restoration of regulatory T cells can prevent inflammatory colitis^[29]. Therefore, natural regulatory T cells, CD4⁺CD25⁺CD45RB^{low}, are considered important cells in maintaining peripheral tolerance. Regulatory T cells are also recognized as playing an important role in cancer diseases^[30]. For gastric^[31,32], esophageal^[33], and other gastrointestinal malignancies, regulatory T cells were increased in peripheral blood. For breast cancer, regulatory T cells were increased in peripheral blood and in the tumor microenvironment^[34]. For lung cancer, regulatory T cells selectively inhibited host immune response and may have contributed to disease progression^[35]. For HCC, CD4⁺CD25⁺ regulatory cells are also found in the tumor by immunohistochemical staining, and the number of regulatory T cells is correlated to the prognosis. In our previous study, regulatory T-cells were identified in the tumor microenvironment. The number of regulatory T-cells was correlated to tumor size and contributed to prognosis. These regulatory T cells also appeared to suppress the DC-mediated

immune responses^[36].

Regulatory T-cells are not the only immunosuppressive cells in hosts with cancer. In a murine cancer model, a group of cells expressing CD11b and Gr-1 in the spleen was noted when the cell population of spleen was analyzed. These cells are currently called myeloid-derived suppressor cells (MDSC)^[37]. Actually, MDSC are a population of cells of myeloid origin, including myeloid progenitors, immature macrophages, immature granulocytes, and immature dendritic cells. MDSC are characterized by production of reactive oxygen, nitrogen species, and arginase I to suppress immunity^[38,39]. The CD11b⁺/Gr-1⁺ cells were expanded in mice bearing large tumors^[40]. Deletion of CD11b⁺/Gr-1⁺ cells *in vitro* or *in vivo* reverses the depression of CD8⁺ T-cell function.

Many researchers attempt to decrease or deplete MDSC to enhance cancer treatment^[41-44]. Kusmartsev *et al.*^[45] implanted slow-releasing all-trans-retinoic acid subcutaneously in order to decrease MDSC from 27% to 11%. De Santo *et al.*^[46] used nitro-aspirin, which released NO to interfere MDSC inhibitory enzymatic activity. Gemcitabine, amino-biphosphonase, and celecoxib all have been used to reduce the number of MDSC in order to enhance cancer treatment^[47]. Moreover, all-trans-retinoic acid has been employed to treat renal cancer patients with metastasis, and there was a clinical response in 1 patient (1/18)^[48]. These data in animal models and a limited number of clinical trials implied that depletion or decrease of MDSC might be helpful in cancer treatment.

CLINICAL CELL-MEDIATED IMMUNOTHERAPY FOR HCC

DC-based immunotherapy

As mentioned, DCs are the most potent antigen-presenting cells. Theoretically, DCs can capture HCC-associated antigens, process the antigens, and activate antigen-specific T-cells to get rid of the tumors. However, the function of DCs is defective in advanced HCC, and antigen-specific T-cells cannot be activated properly. Therefore, DCs are cultured and matured *ex vivo* for immunotherapy.

In a phase I trial, Iwashita *et al.*^[49] enrolled 10 patients to receive autologous DC to treat unresectable HCC. DC was administered by injection into inguinal lymph nodes. Seven of 10 patients experienced delayed-type hypersensitivity response and one patient had tumor response. It was concluded that DC administered was safe and no major toxic effects were found. In another study, Palmer *et al.*^[50] used autologous DC pulsed

with liver tumor cell line lysate (Hep G2) to treat 35 patients with advanced HCC. Twenty-five patients who received at least 3 courses of DC infusion were assessed for tumor response. Disease control rate was 28%. Qiu *et al.*^[51] conducted a phase II clinical trial using α -1,3-galactosyl epitopes pulsed DC to treat stage III HCC patients. They enrolled 9 patients to have DC vaccination and 9 patients as control. The results showed that all patients had delayed hypersensitivity. Three of the 9 patients with DC vaccination had partial response. Compared to control, mean survival was prolonged from 10.1 to 17.1 months^[51]. In our previous study, DC progenitors from peripheral blood monocytes (PBMC) were cultured in granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4, pulsed with autologous tumor lysates and matured by cytokine cocktail (tumor necrosis factor- α , IL-1, IL-6 and PGE2). These DCs were positive for CD83 and expressed high levels of CD40, CD80, CD86 and HLA-DR. In *in-vitro* study, these DCs could activate T-cells. These *ex-vivo* prepared DCs were applied intravenously to treat 31 patients with advanced HCC. Among 31 patients, 4 (12.9%) patients had partial response and 17 (54.8%) patients had stable diseases. Disease control rate was 67.7%. Compared to the same stage patients without DC treatment, the 1- and 2-year survival rates were significantly prolonged^[25]. DC can be injected into the tumors directly. Nakamoto *et al.*^[52] enrolled 10 patients to receive autologous DC infusion into tumors following TACE. DC could be detected in the tumors for up to 17 days when the DCs were labelled with ¹¹¹Indium. Tumor antigen-specific lymphocytes could be found around the tumors. However, no clinical benefit was found in this study.

Checkpoint inhibitor immunotherapy

Activation of naïve T-cells by DC is through ligation of MHC class I/II and T-cell receptor (signal 1) and costimulatory molecular pathways (signal 2). Costimulatory molecular pathways may deliver positive or negative signals to T-cells and result in T-cell activation or T-cell anergy to specific antigens. In immunotherapy of cancer, checkpoint inhibitors can block negative costimulatory molecular pathways and enhance T-cell-mediated immunity. Clinically, checkpoint inhibitor immunotherapy already has obtained promising results in treating advanced melanoma. Tremelimumab, a blockade of cytotoxic T-cell antigen 4 (CTLA-4), was used to treat hepatitis C patients with HCC in a clinical trial^[53]. Twenty patients were enrolled and 17 patients were available to assess therapeutic responses. Partial response rate was 17.6%, stable disease was 76.4%, and time to progression was 6.48 months. Nivolumab, anti-

programmed cell death protein-1, is another checkpoint inhibitor employed in a clinical trial of HCC treatment. In total, 262 patients were enrolled in dose-escalation and dose-expansion phases. The objective response rate was 20%. Complete response rate was 1%, partial response was 18%, and stable disease was 45%. The median progression-free survival was 4 months^[54]. Currently, several clinical trials of checkpoint inhibitor immunotherapy are ongoing. Results will be published in the near future.

T-cell immunotherapy

T-cells are direct effector cells to attract cancer cells. Activated T-cells can be applied to treat cancers^[55]. Takayama *et al.*^[56] conducted a randomized clinical trial by infusion of T-cells to prevent HCC recurrence after curative resection of HCC. One hundred and fifty patients were enrolled and randomized: 76 received adoptive immunotherapy with activated T-cells, and 74 patients received no adjuvant treatment. Autologous lymphocytes were activated by recombinant IL-2 and anti-CD3 antibody and could be expanded more than 1000-fold. Compared to control, the frequency of tumor recurrence was decreased by 18%, and time to the first tumor recurrence was longer. However, overall survival was not significantly affected.

Jiang *et al.*^[57] conducted a phase I trial using autologous tumor-infiltrating lymphocytes (TIL) to prevent tumor recurrence after curative resection for HCC. TIL was obtained from adjacent-tumor tissue and could be expanded by IL-2 and anti-CD3 in 15 of 17 patients. When the expanded TIL was infused back into the patients, only grade 1 flu-like symptoms and malaise were noted. After a median follow-up of 14 months, 12 patients were tumor-free and 3 patients had tumor recurrence. Basically, immunotherapy with expanded autologous TIL was safe and the toxicity was low. Future clinical trials may be conducted by the authors.

Cytokine-induced killer cell therapy

Autologous cytokine-induced killer (CIK) cells were also used to provide cell-mediated immunotherapy for HCC. Shi *et al.*^[58] conducted a phase I clinical trial using CIK cells to treat HCC. CIK cells were expanded from PBMC *ex vivo* by interferon- γ in the first day and followed by anti-CD3, IL-1 α , and IL-2. After CIK cells were infused, the populations of CD8⁺ cells and DCs were both increased. Tumor volume was decreased in 3 of 13 patients. The authors concluded that infusion of CIK cells was safe and immunological status could be improved. Since CIK cells might show anti-tumor activity for HCC, Hao *et al.*^[59] conducted a randomized trial to compare the treatment efficacy of combination of TACE and CIK cells infusion vs. TACE alone for

unresectable HCC. They enrolled 72 patients treated by combination of TACE and CIK cells infusion and 74 patient treated by TACE alone. The results showed progression-free survival and overall survival were both improved. The 1- and 2-year survival were 71.9% and 62.4% for combination therapy of TACE and CIK cells infusion, compared to 42.8% and 18.8% for therapy with TACE alone. Su *et al.*^[60] collected 7 randomized controlled trials and one controlled clinical trial to perform a meta-analysis study of comparison between DC + CIK cells + TACE/RFA treatment for HCC and control. A total of 349 patients had DC + CIK cells + TACE/RFA treatment, compared to 344 patients as control. The results showed that DC + CIK cells + TACE/RFA treatment improved 1- and 2-year overall survival.

Tumor neo-antigens

A successful DC-based immunotherapy for cancers needs specific tumor-associated antigens to promote anti-cancer immunity. HCC-associated antigens were well reviewed by Hong and Huang^[61] and Sun *et al.*^[62]. Among the reported antigens, α -fetoprotein, glypican-3 (GPC3), and multidrug resistance-associated protein-3 (MRP-3) were frequently expressed on HCC and were employed as tumor antigens to conduct clinical trials. However, the clinical responses were not satisfactory. Recently, Aref *et al.*^[63] found HCA519/TPX2 was an HCC-associated antigen. When DCs were pulsed with this peptide, cytotoxic T-cells could be activated. Zhu *et al.*^[64] found the levels of cytokeratin (CK) 10 in HCC cell lines were higher than in normal liver tissue. CK 10 is a potentially targetable tumor-associated antigen. Whether these antigens can be presented by DC to enhance anti-cancer immunity needs to be proved by clinical trials.

CONCLUSION

HCC is an aggressive cancer and can recur even when tumors are completely removed. Effective treatments for advanced stage HCC are still lacking. Cell-mediated immunotherapy is an attractive therapy for HCC with few toxicities. However, tumor response rates are only around 20% because immunosuppressive factors or cells interfere with the effects of immunotherapy. The combination of increasing immunity and depleting immunosuppressive factors shows promise for future success in conducting cell-mediated immunotherapy for HCC.

DECLARATIONS

Authors' contributions

W.C. Lee contributed solely to the paper.

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Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

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