

REVIEW ARTICLE

Immunotherapy for hepatocellular carcinoma following thermal ablation

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Summary

Hepatocellular carcinoma (HCC) is the fifth most common malignant disease and the third leading cause of cancer-associated deaths worldwide, and is characterized by a dismal prognosis. Despite the significant improvements achieved in liver transplantation, surgical resection, transcatheter arterial chemoembolization (TACE) and local ablative therapies such as percutaneous ethanol injection (PEI), radiofrequency ablation (RFA) and microwave ablation (MWA), the 5-year survival rate still remains unsatisfactory. The main reason is the high recurrence and metastasis rates after treatment. Immunotherapy based

on the stimulation of antitumor immune response could represent a new strategy to control HCC recurrence and metastasis. Liver is an immunity organ itself and thermal ablation promotes the release of tumor-associated antigens. Based on the immune status of HCC patients before and after thermal ablation and tumor-associated antigens, this mini-review concisely discusses the potential of immunotherapy in HCC.

Key words: hepatocellular carcinoma, immunotherapy, thermal ablation, tumor-associated antigens

Introduction

HCC is the fifth most common cancer and the third most common cause of cancer-related deaths worldwide [1]. The incidence of HCC is rising, attributed to HBV and HCV-associated cirrhosis, excessive alcohol consumption and aflatoxin B1 contamination. Despite the significant improvement in liver transplantation, surgical resection, TACE and local ablative therapies such as percutaneous ethanol injection, RFA and MWA, the 5-year survival still remains discouraging, with high recurrence and metastasis rates of HCC as the main problem postoperatively [2]. HCC patients are notoriously resistant to chemotherapy and radiotherapy. In view of these facts, new strategies are required. Immunotherapy aims to provide a more efficient and selective targeting of tumor cells by inducing or boosting the existing tumor-specific immune response [3]. Liver is an immunity organ itself. Thermal ablation not

only reduces tumor load, but also can minimize immunosuppression created by the tumor by enhancing release and exposure of tumor-associated antigens from the dead and apoptotic tumor cells, which further might help overcome the immune tolerance towards the tumor [4,5]. Thermal ablation mainly includes RFA and MWA. RFA creates an alternating electric field within the tissue that causes agitation of the ions present in the target tissue that surrounds the electrode, resulting in frictional heat around the electrode [6,7]. MWA refers to all electromagnetic methods of inducing tumor destruction by using devices with frequencies ≥ 900 MHz [8]. The rotation of dipole molecules accounts for most of the heat generated during MWA [9-11]. The key aim of thermal ablation is to achieve and maintain a temperature range 50 to 100°C throughout the entire target volume, by causing almost instantaneous protein coagulation with irreversible damage to mitochondria and cytosolic cell enzymes. Therefore, such im-

mune-stimulating therapeutic interventions in combination with immunotherapy strategies represent a promising future approach for HCC treatment [12-14]. Relevant studies imply a central role of the combination of immunotherapy and thermal ablation in treating HCC.

Thermal ablation improved the antitumor immune status of HCC patients

The impaired antitumor immune status in HCC patients

The antitumor immune status in peripheral blood and especially in tumor local tissue of HCC patients is significantly lower compared to normal people. Infiltration of T cells in the tumor microenvironment is an important regulator of cancer control. It was reported that the infiltration of CD4⁺/CD25⁺ Tregs in HCC tissues was correlated with an increase in tumor size [9,15]. An abundant accumulation of Tregs concurrent with significantly reduced infiltration of CD8 T cells was found in tumor regions compared with no tumor regions. Expression of granzyme A, granzyme B and perforin was decreased dramatically in tumor-infiltrating CD8 T cells. Furthermore, Tregs of HCC patients inhibited proliferation, activation, degranulation and production of granzyme A, granzyme B and perforin of CD8 T cells induced by antiCD3/CD28 antibodies [16]. Other studies implied that low CD8⁺ T cell counts and high Treg numbers were correlated with poor prognosis in HCC patients [17-19]. Researches have reported that the immunity indexes, such as natural killer cells (NK), lymphokine activated killer cells (LAK) and interleukin-2 (IL-2), decreased sharply in HCC patients, while, in contrast, the proinflammatory cytokines, such as TNF- α , IFN- γ , CXCL10, CCL5 and CCL2, increased significantly [20]. In addition, high neutrophil/lymphocyte ratio was reported as poor prognostic indicator after treatment of HCC, and it reflects HCC recurrence after liver transplantation, may be affected by the inflammatory microenvironment [21].

Thermal ablation promotes the immune function of lymphocytes

The number of CD3⁺ and CD4⁺ T cells, and the activity of NK and LAK were found significantly increased after local thermal ablation, while the number of CD8⁺ T cells decreased in peripheral blood. The volume of CD3⁺, CD45RO⁺, CD56 and CD68 immune cells was enlarged, and the ratio of

CD4⁺/CD8⁺ increased in cancerous tissue, which could last for more than 30 days after local thermal ablation [22].

Thermal ablation improves the immune function of red blood cells

In 1981 Siegel et al. reported that red blood cells possess immune function. It is estimated that 95% of C3b receptors in circulation are located on red cells and that an antigen-antibody-complement complex has 500-1000 times greater chance of being removed from the circulation by a red cell than by a white cell. The fact that red cells also adhere to autologous thymocytes and T cells suggests that they may act as intermediaries bringing antigens and T cells together [24]. The number of C3b decreased in HCC patients, while it increased significantly after local thermal ablation, indicating the immune function of red blood cells was improved.

Thermal ablation decreases the level of immune suppression

Thermal ablation could inhibit the secretion of soluble IL-2 receptor, an immunity inhibitor, from tumor cells, which neutralizes the secretion of IL-2 from lymphocytes. Transforming growth factor- β 1 in peripheral blood, which inhibits the immunological integrity of HCC patients, was found increased 1 week after local thermal ablation, and decreased afterwards [22].

Thermal ablation promotes the release of tumor-associated antigens

Tumor-associated antigens are a prerequisite for the development of immunotherapy. Up until now, several HCC-specific tumor-associated antigens that are targeted by T cells have been identified.

α -fetoprotein (AFP)

AFP is currently the best studied and most promising target antigen for HCC immunotherapy. AFP has immunosuppressive effect and is a characteristic protein secreted by liver cancer cells. It can restrain the differentiation and maturation of dendritic cells (DC), and induce apoptosis. AFP-specific epitopes can induce low to moderate CD8⁺ T cell responses in peripheral blood mononuclear cells (PBMCs) of HCC patients. It has been argued that AFP-specific T cell responses are suppressed and thus difficult to activate [24,25].

Immune cell infiltration in cancerous tissue is negatively correlated with serum AFP level, while the AFP levels decrease back to normal after local thermal ablation in the majority of the patients.

NY-ESO-1

Expression of NY-ESO-1 is limited to testis in healthy people, however, it is often expressed *de novo* in HCC and other cancers. NY-ESO-1 expression is associated with worse HCC outcome following treatment, and the mechanism may be that NY-ESO-1 increases tumor cell migration [26,27].

SSX-1 & 2

SSX-1 might be a new potentially promising target in HCC. High specific expression of SSX-1 mRNA could be used as indicator for short-term recurrence, metastasis and poor prognosis [28]. SSX-2 is naturally immunogenic as demonstrated in HLA-A2(+) melanoma patients [29]. In 2 of 6 HLA-A2(+) HCC patients, SSX-2-specific CD8 (+) T cells naturally responded to the disease, because they were enriched in tumor tissues but not in non tumoral liver [30].

Glypican-3 (GPC3)

GPC3 is a foetal oncoprotein which belongs to the family of heparan sulphate proteoglycans that can bind growth factors and thereby promote tumor growth. GPC3 overexpression indicates a poor prognosis for patients with HCC, and it may also have predictive potential for HCC invasion and metastasis [31,32].

Telomerase reverse transcriptase (TERT)

TERT is a catalytic enzyme required for telomere elongation and its expression correlates with telomerase activity. Tumors must retain the length of their telomeres to protect them from degradation. It has been reported that 80-90% of HCC express TERT [33]. TERT mRNA expression in peripheral blood may not be a useful prognostic markers for HCC [34].

Perspectives for immunotherapy for HCC

Cytokine therapy

The principle of cytokine therapy *in vivo* is to adjust and strengthen the function of immune cells to exert a more efficient antitumor immune activity. IL-2 is a growth factor that stimulates

the proliferation of cytotoxic T cells, helper T cells, NK cells, and LAK cells, all of which can participate in the antitumor response [35]. Many HCC animal models demonstrated that local secretion of IL-2 abrogated the tumorigenicity of cytokine-producing tumor cells and inducing a long-lasting protective immune response against a subsequent tumor graft [36,37]. Interferon (IFN) therapy can decrease the levels of ALT and AFP levels which were significantly associated with hepatocarcinogenesis. IFN was also shown to be useful in improving the prognosis of HCC [38].

Peptide vaccines therapy

AFP represents an useful marker for HCC and for monitoring patients' response to therapy, and has immunosuppressive properties. The characterization of AFP-immunogenic epitopes gives the opportunity to design AFP-based cancer vaccines. The activity of AFP-based vaccines has been investigated as a potential tool for cancer therapy [39]. The GPC3 antigen is an ideal target of anticancer immunotherapy against HCC. The GPC3 peptide vaccine induced a GPC3-specific cytotoxic T lymphocytes (CTLs) response which was correlated with overall survival (OS). In this study, OS was significantly longer in patients with high GPC3-specific CTL levels than in those with low levels [40,41].

Adoptive immunotherapy

Adoptive immunotherapy is the administration of special or nonspecial antitumor cells, mainly T cells, to patients for improving their antitumor immunity and prognosis. In their study Kanai et al. reported that T cells were co-cultured with IL-2, anti CD3 monoclonal antibody and special or nonspecial antigens to induce, activate and amplify the T cells. Adoptive immunotherapy includes administration of LAK cells, cytokine-induced killer (CIK) cells, tumor-infiltrating lymphocytes (TILs), CTL and NK T cells (NKT). LAK cells are peripheral blood monocyte cells activated by high concentration of IL-2. Reduction of tumor size was observed after LAK therapy in HCC [42]. TILs are lymphocytes from tumor tissue or malignant effusions (ascites, pleural effusion) which can be activated and amplified *in vitro* by co-culture with IL-2. The number of TILs was significantly correlated with good prognosis [43]. CIK cells were shown to be a heterogeneous population, the majority of which expresses both the T-cell marker CD3 and NK cell marker CD56. CTL

and CIK cells are effective antigen-specific and non-specific tumor-killing effectors. Several clinical studies showed that adoptive immunotherapy can decrease the hepatitis viral load, improve hepatic function and the HCC prognosis [14,44-46].

Conclusions

In conclusion, tremendous progress has been made in the field of immunotherapies for HCC in recent years. However, T cell response generated by immunotherapy in HCC is the most important

topic. Therefore, more attention should be paid to the specific T cell immunotherapy. Improved results in HCC will derive by combining thermal ablation with passive and active immunotherapy.

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