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Potential of Radiofrequency Ablation in Combination with Immunotherapy in the Treatment of Hepatocellular Carcinoma

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Abstract

Radiofrequency ablation (RFA) is an important treatment option for patients with early hepatocellular carcinoma (HCC). RFA offers a reliable, reproducible modality to effectively treat hepatic lesions with minimal collateral damage to the surrounding hepatic parenchyma. In addition to traditional open operative techniques, RFA can be performed percutaneously or laparoscopically to minimize the physiologic insult to the patient. Due to the concomitant hepatic damage and dysfunction that often is present in patients with HCC these factors make RFA a frequently utilized therapeutic option. However, RFA is most efficacious in treating smaller tumors (≤ 2 cm), particularly when an ablation margin of ≥ 4–5 mm can be obtained. RFA has diminishing utility in larger tumors, resulting in reduced three and five year overall survival rates when compared to surgical resection. Multimodal approaches to include RFA with other standard and investigational approaches have become a subject of recent interest. RFA capably produces cellular destruction causing liberation of a substantial amount of antigens, many of which are tumor-specific providing a favorable environment for immune recognition. We propose that utilizing an immunotherapeutic approach in conjunction with RFA is the next logical step in the treatment of HCC. In this review, we summarize how RFA modulates antitumor immunity and works in concert with immunotherapy in the treatment of HCC. The information provided is expected to help the future design of novel RFA-integrated immunotherapies which are able to generate durable and powerful antitumor immune response to achieve optimal tumor control.

Keywords

Hepatocellular carcinoma (HCC); Radiofrequency ablation (RFA); Immunotherapy; Cancer; Integration

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Introduction

Hepatocellular carcinoma (HCC) is the most rapidly increasing type of cancer in the United States (US) [1]. This is due to the prevalence of viral hepatitis and other causes of hepatic cirrhosis [2]. Worldwide, HCC is the third leading cause of cancer death and in the US HCC accounts for 14,000 deaths per year [3]. HCC is refractory to traditional chemotherapies, and surgical resection or ablation offers a small chance for cure. Unfortunately, most patients are not candidates for surgical resection or even ablation due to factors related to the primary tumor and/or associated comorbidities. Though liver transplant is an effective therapy in some cases, donor organs are scarce and often patients are not candidates for transplantation [4].

In 2008, the receptor tyrosine kinase inhibitor (RTKI), sorafenib, became the first and only systemically administered therapy to show efficacy in the treatment of unresectable HCC. It was thus approved by the Food and Drug Administration (FDA) to treat advanced HCC, as it increased the median overall survival from 7.9 to 10.7 months [5]. This small but statistically significant therapeutic effect highlights the challenge in treating this devastating disease. A commonly utilized method of treating HCC is with radiofrequency ablation (RFA). RFA is a minimally invasive, reliable, and reproducible procedure with few complications [6]. RFA has gained worldwide acceptance for treatment of primary hepatic tumors or metastases [7]. This approach often can be applied through open surgical, laparoscopic, or percutaneous techniques. RFA can target the tumor within the parenchyma through image guidance and spare the normal surrounding liver [8]. Treatment outcomes indicate that RFA is a safe and efficient method of tumor ablation with low morbidity and mortality rates, and maximal perseveration of normal liver parenchyma [9]. RFA is most efficacious in treating smaller tumors (≤ 2 cm), particularly when an ablation margin of ≥ 4–5 mm can be obtained [10]. RFA has diminishing utility in larger tumors, resulting in reduced three and five year overall survival rates when compared to surgical resection [11].

Immunotherapy represents the most promising new approach in the treatment of cancers [12]. For HCC or any cancer to progress it must evade the immune system; in fact, evading immune destruction is now considered a hallmark of cancer [13]. Evidence that immunity plays an important role in mediating cancer control stems from reports of elevated cancer risk in immunosuppressed allogeneic organ graft recipients, and the opportunistic growth of virally induced lymphomas and Kaposi’s sarcoma in patients with HIV-AIDS. Additionally, RAG and STAT knockout mice, which lack mature lymphocytes, are more susceptible to chemically induced cancers [14]. It is now clear that even after cancer develops, the immune system can be harnessed to destroy tumor cells [15]. The antibody-mediated blockade of cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) were recently approved by the US FDA in the treatment of patients with advanced melanoma [16] and squamous non-small cell lung cancer [17]. These recent developments support the translation of immunotherapies to other cancers, but none are currently approved for the treatment of HCC.

RFA monotherapy has been verified to change the number, phenotype and function of different immune cell subsets which are correlated with patient survival [18]; however, this

effect is not sufficient to prevent tumor recurrence and metastasis completely. Therefore, integration of various immunotherapeutic modalities with RFA toward powerfully activating antitumor immunity may potentially improve therapeutic efficacy and has been demonstrated by numerous studies [19]. In this review, we summarize how RFA modulates antitumor immunity and works in concert with immunotherapy in the treatment of HCC. The information provided is expected to help future design of novel RFA-integrated immunotherapies which are able to generate durable and powerful antitumor immune response to achieve optimal tumor control.

Relationship of RFA-Mediated Antitumor Immunity with Tumor Suppression and Patient Prognosis

It is now clear that the immune system can recognize and respond to tumor cells either in their normal environment or following therapeutic intervention [13]. Interaction of tumors and host immunity is required to develop an effective antitumor immune response. In this process, tumor antigens can be directly captured by lymphocytes in lymph nodes or delivered by dendritic cells (DCs) on MHC molecule of T cells [20]. In an ideal circumstance, effector immune cells are successfully activated and exert cytotoxic function to eradicate the tumor cells and also develop tumor-specific immunologic memory to prevent further tumor progression [21]. However, tumor development creates a tumor microenvironment (TME) which can impair the host immune system in diverse ways via various mechanisms. This results in a tolerant TME that not only protects tumor cells from immune elimination but also promotes tumor growth [22]. The development of this environment suggests that the host immune system plays a vital role in the defense against cancer development and growth.

RFA has emerged as an important standard of care treatment option for patients with early HCC according to the Barcelona Clinic Liver Cancer group (BCLC) [23]. This technique is designed to induce tumor destruction by delivering a high frequency alternating current through an active needle-electrode introduced into the neoplastic tissue [24]. The resultant ion movement generates frictional heating which causes cell death by means of coagulative necrosis. At 43°C apoptosis is seen in 30–60 sec; cellular death occurs in a few minutes at 50°C, in a few seconds at 55°C, and almost instantaneously at temperatures above 60°C [25]. An increasing number of studies demonstrate that RFA treatment releases tumor antigen to prime the immune system and, at least in part, impacts antitumor therapeutic efficacy.

The first study to identify RFA-mediated antitumor response was conducted in 2003 by Wissniowski et al. [26]. After treating an induced liver cancer in rabbits with RFA, they harvested lymphocytes and the liver tissue to conduct immunohistochemical analysis. The results suggested that a tumor-specific T-cell response was elicited 2 weeks after RFA treatment. The treated cohort displayed a prolonged survival despite high recurrence rates. This suggested a substantial immunological effect on tumor growth.

Very recently, Wissniowski et al. used a VX2 rabbit hepatoma model to assess RFA-mediated antitumor immune response and therapeutic efficacy [27]. Compared with

untreated controls, RFA was found to induce a 26-fold increase in antitumor T-cell stimulation and a 16-fold increase in antitumor cytotoxicity. Correspondingly, the mean survival of RFA-treated mice was 97 days which is significantly longer than the 36 days observed in the untreated mice. Integration of the toll-like receptor 9 agonist, CpG, with RFA enhances antitumor T-cell stimulation 50-fold and antitumor cytotoxicity 38 fold, respectively. As a result, this combinational treatment prolonged mean survival to 114 days and significantly inhibit tumor spread to the lungs and peritoneum; in addition, this strategy prevented new tumor growth in animals receiving a secondary systemic tumor-cell injection.

Another recent study, demonstrated that the number of tumor associated antigen (TAA)-specific T cells after RFA could predict the recurrence of HCC [18]. The results suggest that the number of TAA-T cells after RFA was positively correlated with the recurrence-free survival of HCC patients but inversely correlated with the frequency of $CD14^{+}HLA\text{-}DR^{-/low}$ myeloid-derived suppressor cells (MDSCs). However, the memory phenotype and lifetime of TAA-T cells are not sufficient to prevent HCC recurrence completely. Geier et al., investigated the effect of single RFA treatment in a two-tumor rat model of HCC [28]. The results showed that RFA treatment on the left lobe tumor enhanced T cell frequency and IL10 production in the non-RFA-targeted tumor on the right lobe and suppressed its growth. Another group demonstrated that in situ tumor ablation with RFA creates an antigen profile which is able to induce a weak but tumor-specific immune response [29]. They also demonstrated that the observed immune response is mainly cell mediated and can be transferred into other mice.

Napoletano et al., reported that RFA strongly modulated the immune system [30]. Following treatment RFA, thirteen patients with metastatic liver lesions and four patients with HCC were observed the increased trafficking of naïve and memory $CD62L^{+}$ T cells, which is accompanied with activation of T cells, evidenced by IFN-γ production in response to MUC1 antigen.

Despite a relatively small amount of evidence to show the relationship of RFA-induced immune activation and therapeutic outcome, the current studies demonstrate that the immune response after RFA or RFA-integrated therapies involves tumor suppression and subsequently determines the prognosis of HCC patients and tumor-bearing mice.

RFA Treatment Promotes Antitumor Immune Response

It is well-known that tumor growth impairs antitumor immunity in HCC patients which is one of the mains causes inducing tumor recurrence and metastasis after different treatments. Present studies demonstrate that treatment of HCC patients with RFA generates heat shock and massive necrotic cell death which primes the tumor antigen-specific immune response [19]; however, despite this tumor recurrence and metastasis frequently occurs.

In 2004, Gosse et al. demonstrated that in situ tumor ablation with RFA is able to induce a weak but tumor-specific immune response [29]. As previously discussed, this observed immune response is mainly cell mediated and can be transferred from RFA-treated mice into naive mice via splenocytes. In 2006, Missale et al. reported that RFA activates the tumor-

specific T-cell response in patients with HCC [31]. In this study, peripheral blood mononuclear cells (PBMCs) were isolated from 20 HCC patients before and one month after RFA treatment. A significant increase in the frequency of circulating tumor-specific T cells and their IFN-γ production in PBMCs was detected after RFA treatment. However, tumor-specific T-cell responses were not associated with protection from HCC relapse; one patient showed immune escape evidenced by occurrence of a new tumor nodule which was not recognized by T cells obtained at the time of RFA. Geissler et al. demonstrated that treatment of HCC patients with RFA induced a functional transient activation of myeloid dendritic cells (DCs) but not plasmocytoid DCs, which is associated with increased levels of TNF-α and IL-1β [32]. Schuppan et al., used rabbits to make an orthotopic HCC model by implanting VX2 hepatoma cells [26]. Treatment of tumor-bearing rabbits with RFA resulted in the activation of circulating T cells in response to tumor antigen stimulation. This activation lasted 45 days after treatment and was accompanied with dense T-cell infiltration. In contrast, T cells in untreated tumor-bearing rabbits showed no reaction and only sparse T cell infiltration. In 2012, Nakatsura et al. compared the immune response against TAA GPC3 in HCC patients and tumor-bearing mice with or without RFA treatment [33]. The results indicated that circulating GPC3-specific cytotoxic T lymphocytes (CTLs) were increased in 5 out of 9 patients exposed to RFA treatment. All patients with GPC3-expressing HCCs exhibited an increase in GPC3-specific CTLs in RFA-treated patients compared to surgical resection. Similar results were detected in tumor-bearing mice. In 2013, Kaneko et al. analyzed the immune response against 11 TAA-derived peptides in 69 HCC patients. Enzyme-linked immunospot (ELISPOT) and tetramer assays detected an increased number of diverse TAA-specific T cells in 62.3% of patients after RFA treatment.

In conclusion, RFA-generated in situ tumor destruction can provide a useful antigen source for the induction of antitumor immunity. While this effect is not sufficient for controlling HCC, it may represent the underpinnings for the development of RFA-integrated immunotherapy in patients.

RFA in Combination with Additional Immunotherapeutic Strategies in the Treatment of HCC

Although RFA augments antitumor immune response in HCC patients, the frequent postoperative recurrence and metastasis of tumors suggest that the RFA-induced tumor-specific immune response is not sufficient. Thus, additional treatment modalities which will further strength anti-tumor immunity may optimize RFA for liver malignancies.

Yoon et al., performed a multicenter, randomized, open-label, phase 3 trial of the efficacy and safety of adjuvant immunotherapy with activated cytokine-induced killer (CIK) cells in 230 HCC patients (NCT00699816) [34]. Following treatment with surgical resection, RFA, or percutaneous ethanol injection, half of the patients were randomly assigned to receive immunotherapy. They reported that the median time of recurrence-free survival was 44 months in the immunotherapy group and 30 months in the control group with no significant serious adverse events.

Kaneko et al., made a xenograft mouse model with a murine HCC hepatoma cell line [35]. The generated tumor-bearing mice received RFA treatment with or without injection of ECI301, an active variant of CC chemokine ligand 3. They demonstrated that a single RFA treatment inhibited the growth of contralateral non-RFA-treated tumors and was accompanied by massive T-cell infiltration. Injection of ECI301 augmented RFA-induced antitumor effect against non–RFA-treated tumors with increased $CD11c^+$ cells in peripheral blood and also increased IFN-γ production, resulting in enhanced antitumor immune responses.

Adema's research team developed a tumor model with the murine B16-OVA melanoma cell line. Using this model, they investigated the immunologic consequences of RFA-mediated in situ tumor destruction and the combined effect on tumor destruction and immunostimulation with injection of antibodies against CTLA4 [29]. They found that RFA alone induces a weak but detectable immune response against OVA. In contrast, injection of anti-CTLA4 at the time of RFA generates a strong antitumor immunity, resulting in long-lasting tumor protection.

Wissniowski et al., tested the antitumor T cell response and cytotoxicity in the rabbit VX2 hepatoma model in response to the combined treatment with TLR9 stimulation and RFA [27]. They demonstrated that only this combination prevented subsequent tumor spread and resulted in a significantly improved survival, justifying the further exploration of the combination of RFA and TLR9 agonists in HCC.

Kaneko et al., investigated the immune response before and after RFA in 69 HCC patients using HCC TAA peptides [18]. This study found that despite the diversity of the induced antitumor immune response, the frequency of RFA-induced TAA-specific T cells is positively related with the prevention of tumor recurrence.

Li et al., utilized combined therapeutic strategy to treat HCC patients [36]. The autologous mononuclear cells were harvested from 30 patients, and induced into natural killer (NK) cells, γδT cells and cytokine-induced killer (CIK) cells. These cells were then infused intravenously to RFA-treated patients for three or six courses. The results implied that progression-free survival (PFS) was higher in combinational group than that in RFA monotherapy group (32 patients).

Kaneko's team used colon cancer cells to make a bilateral flank orthotopic murine tumor model [37]. Using this model, they investigated the in vivo immunological antitumor effect of OK-432-stimulated DCs following RFA treatment on contralateral tumors. Compared to RFA alone, the combination of RFA and DC transfer strongly inhibited contralateral tumor growth.

Collectively, RFA monotherapy is able to induce antitumor activity, but it is insufficient to prevent tumor recurrence. Additional immunotherapeutic strategies are required to further strengthen the HCC-specific immune response in order to more effectively treat HCC.

Future Perspectives

The prospect of manipulating the immune system toward the rejection of established cancers as part of the standard of care of patients is becoming closer to reality. RFA has been applied to clinical practice and has rapidly become an important first-line therapy in the treatment of HCC. In situ tumor ablation with RFA releases numerous tumor antigens which can prime a tumor antigen-specific immune response; however, this effect is not sufficient to completely treat the tumors. Integration of additional immunotherapeutic strategies with RFA is expected to generate durable and powerful antitumor immunity to achieve optimal tumor control.

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