

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

Guangfu Li^{1,2*}, Kevin F Staveley-O'Carroll^{1,2,3*} and Eric T Kimchi^{1,2*}

¹Department of Surgery, University of Missouri, Columbia, MO 65212, USA

²Ellis Fischel Cancer Center, University of Missouri, Columbia, MO 65212, USA

³Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65212, USA

*Corresponding authors: Guangfu Li, Department of Surgery, Ellis Fischel Cancer Center, The University of Missouri, One Hospital Drive, Columbia, MO 65212, USA, Tel: +1-573-882-8454; Fax: +1-573-884-6054; E-mail: liguan@health.missouri.edu

Kevin F Staveley-O'Carroll, Ellis Fischel Cancer Center, The University of Missouri, One Hospital Drive, Columbia, MO 65212, USA, Tel: +1-573-882-8454; Fax: +1-573-884-6054; E-mail: ocarrollk@missouri.edu

Eric T Kimchi, Ellis Fischel Cancer Center, The University of Missouri, One Hospital Drive, Columbia, MO 65212, USA, Tel: +1-573-882-8454; Fax: +1-573-884-6054; E-mail: kimchie@health.missouri.edu

Received date: January 20, 2016; Accepted date: March 29, 2016; Published date: April 05, 2016

Copyright: © 2016 Li G, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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 $\geq 4-5$ 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

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 preservation of normal liver parenchyma [9]. RFA is most efficacious in treating smaller tumors (≤ 2 cm), particularly when an ablation margin of $\geq 4-5$ 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 Wissniewski 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, Wissniewski 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-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 main 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 plasmacytoid DCs, which is associated with increased levels of TNF- α and IL-1 β [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.

Wisniewski 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, $\gamma\delta$ T 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.

Acknowledgement

Grant Support: 1 R01 CA164335-01A1 (Kevin F. Staveley-O'Carroll, PI) from the National Cancer Institute/National Institutes of Health; In part by an ACS-IRG pilot research funding from an American Cancer Society Institutional Research Grant awarded to the Hollings Cancer Center, Medical University of South Carolina (Guangfu Li, PI). University of Missouri Department of Surgery Grant support (Guangfu Li, PI; Kevin F. Staveley-O'Carroll, PI; Eric T. Kimchi, PI).

References

1. Tanabe KK, Chiocca EA (2013) "Infectious" optimism for treatment of hepatocellular carcinoma. *Mol Ther* 21: 722-724.
2. Arzumanyan A, Reis HM, Feitelson MA (2013) Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 13: 123-135.
3. Yeh MM, Yeung RS, Apisarnthanarax S, Bhattacharya R, Cuevas C, et al. (2015) Multidisciplinary perspective of hepatocellular carcinoma: A Pacific Northwest experience. *World J Hepatol* 7: 1460-1483.
4. Altekruse SE, McGlynn KA, Dickie LA, Kleiner DE (2012) Hepatocellular carcinoma confirmation, treatment, and survival in surveillance, epidemiology, and end results registries, 1992-2008. *Hepatology* 55: 476-482.
5. Chaparro M, González Moreno L, Trapero-Marugán M, Medina J, Moreno-Otero R (2008) Review article: pharmacological therapy for hepatocellular carcinoma with sorafenib and other oral agents. *Aliment Pharmacol Ther* 28: 1269-1277.
6. Lo GH (2015) Is cryoablation really more effective than radiofrequency ablation in hepatocellular carcinoma? *Hepatology* .
7. Cho YK (2014) A comparison of surgical resection and radiofrequency ablation for the treatment of single small hepatocellular carcinoma \leq 2 cm. *Hepatology* 59: 1653.
8. Ikeda K, Osaki Y, Nakanishi H, Nasu A, Kawamura Y, et al. (2014) Recent progress in radiofrequency ablation therapy for hepatocellular carcinoma. *Oncology* 87 Suppl 1: 73-77.
9. Ng KK, Poon RT (2005) Radiofrequency ablation for malignant liver tumor. *Surg Oncol* 14: 41-52.
10. Lu DS, Yu NC, Raman SS, Limanond B, Lassman C, et al. (2005) Radiofrequency ablation of hepatocellular carcinoma: treatment success as defined by histologic examination of the explanted liver. *Radiology* 23: 954-960.
11. Duan C, Liu M, Zhang Z, Ma K, Bie P (2013) Radiofrequency ablation versus hepatic resection for the treatment of early-stage hepatocellular carcinoma meeting Milan criteria: a systematic review and meta-analysis. *World J Surg Oncol* 1: 190.
12. Smyth MJ, Ngiew SF, Ribas A, Teng MW (2016) Combination cancer immunotherapies tailored to the tumour microenvironment. *Nat Rev Clin Oncol* 13: 143-158.
13. Turley SJ, Cremasco V, Astarita JL (2015) Immunological hallmarks of stromal cells in the tumour microenvironment. *Nat Rev Immunol* 15: 669-682.
14. Mapara MY, Sykes M (2004) Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. *J Clin Oncol* 22: 1136-1151.
15. Mueller KL (2015) Cancer immunology and immunotherapy. Realizing the promise. *Introduction. Science* 348: 54-55.
16. (2015) Pembrolizumab superior to ipilimumab in melanoma. *Cancer Discov* 5: 568.
17. Waqar SN, Morgensztern D (2015) Immunotherapy for non-small cell lung cancer: are we on the cusp of a new era? *Expert Rev Clin Immunol* 11: 871-873.
18. Mizukoshi E, Yamashita T, Arai K, Sunagozaka H, Ueda T, et al. (2013) Enhancement of tumor-associated antigen-specific T cell responses by radiofrequency ablation of hepatocellular carcinoma. *Hepatology* 57: 1448-1457.
19. Chu KF, Dupuy DE (2014) Thermal ablation of tumours: biological mechanisms and advances in therapy. *Nat Rev Cancer* 14: 199-208.
20. Palucka K, Banchereau J (2012) Cancer immunotherapy via dendritic cells. *Nat Rev Cancer* 12: 265-277.
21. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. *Cell* 140: 883-899.
22. Gajewski TF, Schreiber H, Fu YX (2013) Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* 14: 1014-1022.
23. Liu PH, Hsu CY, Lee YH, Hsia CY, Huang YH, et al. (2015) When to Perform Surgical Resection or Radiofrequency Ablation for Early Hepatocellular Carcinoma?: A Nomogram-guided Treatment Strategy. *Medicine (Baltimore)* 9: e1808.
24. Qi X, Shao H, Zhang J, Sun Z, Ni Y, et al. (2015) Radiopharmaceutical study on Iodine-131-labelled hypericin in a canine model of hepatic RFA-induced coagulative necrosis. *Radiol Med* 120: 213-221.
25. McGahan JP, Brock JM, Tesluk H, Gu WZ, Schneider P, et al. (1992) Hepatic ablation with use of radio-frequency electrocautery in the animal model. *J Vasc Interv Radiol* 3: 291-297.
26. Wissniewski TT, Hänslér J, Neureiter D, Frieser M, Schaber S, et al. (2003) Activation of tumor-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits. *Cancer Res* 63: 6496-6500.
27. Behm B, Di Fazio P, Michl P, Neureiter D, Kemmerling R, et al. (2016) Additive antitumor response to the rabbit VX2 hepatoma by combined radio frequency ablation and toll like receptor 9 stimulation. *Gut* 65: 134-143.
28. Erös de Bethlenfalva-Hora C, Mertens JC, Pigué AC, Kettenbach J, Schmitt J, et al. (2014) Radiofrequency ablation suppresses distant tumour growth in a novel rat model of multifocal hepatocellular carcinoma. *Clin Sci (Lond)* 126: 243-252.
29. den Brok MH, Suttmuller RP, van der Voort R, Bennink EJ, Figdor CG, et al. (2004) In situ tumor ablation creates an antigen source for the generation of antitumor immunity. *Cancer Res* 64: 4024-4029.
30. Napolitano C, Taurino F, Biffoni M, De Majo A, Coscarella G, et al. (2008) RFA strongly modulates the immune system and anti-tumor immune responses in metastatic liver patients. *Int J Oncol* 32: 481-490.
31. Zerbini A, Pilli M, Penna A, Pelosi G, Schianchi C, et al. (2006) Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. *Cancer Res* 66: 1139-1146.
32. Ali MY, Grimm CF, Ritter M, Mohr L, Allgaier HP, et al. (2005) Activation of dendritic cells by local ablation of hepatocellular carcinoma. *J Hepatol* 43: 817-822.
33. Nobuoka D, Motomura Y, Shirakawa H, Yoshikawa T, Kuronuma T, et al. (2012) Radiofrequency ablation for hepatocellular carcinoma induces glypican-3 peptide-specific cytotoxic T lymphocytes. *Int J Oncol* 40: 63-70.
34. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, et al. (2015) Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology* 148: 1383-1391.
35. Iida N, Nakamoto Y, Baba T, Nakagawa H, Mizukoshi E, et al. (2010) Antitumor effect after radiofrequency ablation of murine hepatoma is augmented by an active variant of CC Chemokine ligand 3/macrophage inflammatory protein-1alpha. *Cancer Res* 70: 6556-6565.
36. Cui J, Wang N, Zhao H, Jin H, Wang G, et al. (2014) Combination of radiofrequency ablation and sequential cellular immunotherapy improves progression-free survival for patients with hepatocellular carcinoma. *Int J Cancer* 13: 342-351.

-
37. Nakagawa H, Mizukoshi E, Iida N, Terashima T, Kitahara M, et al. (2014) In vivo immunological antitumor effect of OK-432-stimulated dendritic cell transfer after radiofrequency ablation. *Cancer Immunol Immunother* 6: 347-356.