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# Radiofrequency ablation combined with immunotherapy to treat hepatocellular carcinoma: a comprehensive review

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## Abstract

**Background and aim** Hepatocellular carcinoma (HCC) is a highly immunogenic tumor and the third leading cause of cancer-related deaths worldwide with an increasing incidence. Therefore, the combination of immunotherapy with other approaches, such as anti-angiogenic agents and local area therapy, has become a new strategy for HCC treatment.

**Methods** We searched PubMed and Web of Science and extracted publications relating to the radiofrequency ablation (RFA) and immunotherapy. The search terms were: "radiofrequency ablation", "immunotherapy" and "hepatocellular carcinoma", and manual searches of eligible articles from literature reference lists were performed. We then thoroughly reviewed the literature on ablation combined with immunotherapy for HCC, analyzed the relevant mechanism, and explored the safety and effectiveness of this form of combination therapy.

**Results** RFA combined with immunotherapy in HCC is reported to have good efficacy and controllable safety. On the one hand, RFA can induce the immunogenic substances including Ficolin-3, IL-1 and heat shock protein and regulate the immune cells by mediating the Th1/Th2 ratio, increasing Th17 cells, etc. On the other hand, RFA treatment can lead to tumor immune microenvironment reconstruction, increasing the proportion of functional T cells and upregulate PD-1 in T cells in distant tumors without RFA. This combined strategy has the ability to enhance the anti-tumor immune response through synergies, significantly reduce the risk of recurrence and improve survival.

**Conclusions** RFA combined with immunotherapy yields a good synergistic effect: it can further strengthen anti-tumor response, delay distant tumor growth, reduce tumor recurrence and metastasis, providing new options for HCC systemic treatment.

**Keywords** Hepatocellular carcinoma, Radiofrequency ablation, Immunotherapy, Combination therapy

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## Background

Primary liver cancer (PLC) is one of the most common malignancies of the digestive system and globally the third leading cause of cancer-related deaths [1]. Hepatocellular carcinoma (HCC), which accounts for about 90% of all PLCs, is the most common type of primary liver cancer. Most clinical practice guidelines recommend radical resection, ablation or liver transplantation in patients with early-stage HCC (BCLC 0-A), while transarterial chemoembolization (TACE) and systemic therapy are preferred in patients with intermediate (BCLC B) and advanced (BCLC C) HCC, respectively [2–5]. Surgical resection, radiofrequency ablation (RFA) or liver transplantation are the suggested radical treatments for early-stage HCC, and RFA is recommended for solitary tumors less than 2 cm in diameter with a comparable life-expectancy albeit at a lower cost to hepatectomy. However, hepatectomy is not suitable for patients at an advanced stage of the disease and the application of liver transplantation is also limited by strict indications, organ shortages, prolonged cycles, and high costs. Nevertheless, RFA is considered to be one of the best treatment options for patients with more advanced BCLC stages and poorer liver function because of its definite efficacy, local minimally invasive nature, safety, reliability, and reproducibility [6–8].

RFA is a potent treatment strategy for early HCC and the earliest ablation mode applied to minimally invasive treatment for HCC. The principle is to generate a high-frequency current in the tumor by the electrode, so that the electrons around the electrode needle vibrate at high speed, friction with each other to generate more than 60℃ heat in the tumor tissue, achieving tumor tissue dehydration, solidification, protein denaturation, coagulation necrosis, and the final purpose of eliminating the tumor [9, 10]. For example, one study reported that among 187 HCC patients receiving RFA, the 1-year overall survival (OS) rate was 97%, the 3-year OS rate was 71%, the 5-year OS rate was 48%, and the median survival was 57 months [11].

Immunotherapy has shown strong efficacy in patients with various malignancies, including HCC. Among them, the current research trends are immune checkpoint inhibitors such as programmed cell death receptor-1 (PD-1), programmed cell death receptor-ligand 1 (PD-L1), and cytotoxic T lymphocyte associated antigen (CTLA-4) inhibitors. The main principle of these therapies is to inhibit the expression of immunonegative regulators in the tumor microenvironment, thereby enhancing the recognition and processing ability of immune cells to tumor-associated antigen (TAA) and reducing immune escape [12]. The anti-PD-L1 antibody atezolizumab combined with the VEGF neutralizing antibody bevacizumab has resulted in a better long-term prognosis for HCC patients compared to sorafenib [13]. However, the response rate to immunotherapy in solid tumors is generally poor, possibly because these are mostly cold tumors [14]. Thus, the current challenge is to derive strategies to improve the response rate of immunotherapy and transform cold tumors into immunothermal ones. RFA not only kills liver cancer cells but also causes the exposure and release of TAA, enhances the antigenicity of tumors, activates transient immune responses at the local level, and triggers specific anti-tumor cell immunity and systemic immune responses [15, 16]. After RFA, the abundance of CD4 + and CD8 + T cells in the peritumor area and peripheral blood increase significantly [17], and some pro-inflammatory factors and immunostimulating factors also become elevated [18, 19]. Although the effect of this immune response is weak and insufficient to completely prevent HCC recurrence, RFA and immunotherapy can synergistically enhance this immune response [20]. HCC has great heterogeneity and genetic instability; therefore, its pathogenesis is complex. Most HCC patients are in an immunosuppressed state, presenting difficulty to achieve the ideal therapeutic effect via a single treatment modality [21]. RFA can induce autoimmune reaction after treatment, suggesting that it can enhance the effect of immunotherapy by reversing the immunosuppressive state of patients, showing the potential of combined immunotherapy. Combination immunotherapy can further enhance the anti-tumor immune effect of RFA, exert a stronger inhibitory effect on primary tumors and metastases, and effectively reduce tumor recurrence and metastasis. Therefore, the anti-tumor immune mechanism of RFA and its combination with immunotherapy are becoming new research directions.

### Immunosuppressive microenvironment of HCC

The development of HCC is characterized by the interplay of hepatocytes, non-hepatocytes and the immune microenvironment. The main drivers of HCC are cell death pathways, DNA damage and mutations, and inflammatory responses. At the same time, the "metastatic microenvironment" makes the liver the most common target organ of cancer cell metastasis, with a pathological microenvironment triggered by the chronic inflammatory response inhibition of specific antigen immune surveillance being one of the most critical mechanisms, mainly related to the tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and downstream NF-κB, c-Jun N-terminal kinase, and signal transducer and transcriptional activator 3 signaling pathways. With the help of antigen presenting cells, cytotoxic T cells, including CD4+, CD8 + T cells and Th17 cells and B cells, are also involved in initiating the development of HCC [22, 23].

HCC features an immunosuppressive tumor microenvironment (TME), in which tumor cells can promote

the release of molecules with immunosuppressive functions, such as transforming growth factor-β, IL-10, etc. Through endogenous osteopontin, the expression of PD-L1, the expansion of tumor-associated macrophages, and the inhibitory effect of myeloid-derived suppressor cells, a tumor immune microenvironment (TIME) is created that drives tumor cells' immune evasion and the body's immune tolerance [24, 25]. Fujita et al. discovered the immunosuppressive mechanism of HCC by analyzing its genomic data, and immunologically classified HCC types. In the first 'CTNNB1' group, Wnt-β-catenin signaling participated in cell proliferation and differentiation and activated the immunosuppressive mechanism; in the second 'Treg' group, Treg cells were highly expressed and thus enhanced the inhibitory regulatory immune effect; in the third 'TAM' group, M2 macrophages promoted the production of immunosuppressive factors and the infiltration of Treg cells and inhibited the anti-tumor immune effect. The fourth 'high CYT group' had high toxicity for active immunocytes but exhibited no immunosuppressive mechanism [26].

Although the body possesses a variety of immune surveillance mechanisms to exert its anti-tumor immune function, tumor cells can still use a variety of pathways to escape human immune surveillance and killing. Research has shown that the dysregulation of T cell subsets in tumor infiltrating lymphocytes (TILs) in HCC patients can lead to a significant decline in the local immune function of liver tissue [27–29]. Several studies [30, 31] have reported decreased levels of IL-2 and interferon γ (IFN-γ) secreted by helper T cell 1 (Th1) cells in HCC patients, while the levels of IL-4 and IL-10 secreted by Th2 cells increased, resulting in reduced levels of Th1; the Th2 scale shifted towards Th2. The role of Th2 cells in HCC is currently believed to be the promotion of tumor formation. Moreover, the secretion of IL-4, IL-5, IL-10 and other cytokines mediates immunosuppression in patients by inhibiting the differentiation of CD4 + T cells to Th1 cells. Tregs are an important basis for tumor immune tolerance, as they can inhibit the proliferation and activation of CD4 + CD25+, CD8 + T cells, natural killer (NK) cells, and the secretion of IL-2, reduce the efficiency of antigen presentation and promote tumor growth and metastasis [32]. Studies have shown that NK cells are significantly reduced in cancerous tissues compared with non-cancerous ones, which may be due to the secretion of certain factors by tumor tissues leading to the apoptosis of NK cells, thereby evading the body's immune surveillance [33]. Findings indicate that the cellular immunity of HCC patients is in a state of suppression. Therefore, timely and effective intervention is particularly important in the treatment of HCC patients to improve their immune function.

### The mechanism by which RFA regulates immunity in HCC

RFA induces the local coagulation necrosis of tumor cells through thermal effects and the release of antigenic breakdown products, creating an antigen source for anti-tumor immunity (Fig. 1). Inactivated tumor cells act as "vaccines" to induce the production of cytotoxic T cells; their thermal effects can change the spatial structure of antigenic determinants and activate the body's specific immune response. For example, RFA can cause mutations in innate immune cells (NK cells) and adaptive immune cells (CD8 + T cells, CD28 + CD8 + T cells), triggering systemic immune effects [34, 35]. At the same time, it can promote the body's production of heat shock protein 70 and activate the body's autoimmunity to achieve indirect anti-tumor effects [36]. Heat injury can lead to persistent local inflammation, stimulate the body's cellular immune response, improve the patients' immunosuppressive state and promote the recovery of autoimmune function. RFA stimulation reduces the number of soluble IL-2 receptors in HCC patients, which in turn decreases the level of IL-2 secreted by activated T cells. In addition, RFA can promote the secretion of Th1 cytokines and the activation of monocytes, increase the ratio of Th1/Th2 cytokines, activate myeloid dendritic cells, obliterate the body's immunosuppressive microenvironment, and enhance the immune function of lymphocytes [37]. Zerbini et al. found that cell fragments released from tumor tissue after RFA contribute to the maturation of antigen-presenting cells and dendritic cells (DCs) in vivo, further promoting the activation of the immune system [34]. Increased plasma levels of IL-1, IL-6, IL-8, and TNF-α have been reported after RFA treatment [38]. Significant morphological changes were observed in tumor tissues after RFA, which could be characterized by four regions: needle zone, central zone, transition zone, and marginal zone [39]. High temperature from RFA was reported to induce cell necrosis and increase heatshockprotein-70 (HSP-70) expression mainly in the transition region [40]. Increased expression levels of HSP-70 and HSP-90 induced by RFA treatment were also observed in animal tumor models [41]. Furthermore, HSP-70 was demonstrated to play a key role in the activation of innate and adaptive immune cells [42]. After RFA, the expression of HSP-70 in paracancer tissues is increased and the infiltration and activation of NK cells are induced, which constitute the innate immune response induced by RFA [43].

### Mechanism of the anti-tumor effect of RFA

The anti-tumor immune effect of RFA is mainly manifested in two aspects: (1) Release of immunogenic substances: the destruction of cell structure caused by high temperature will release a variety of immunogenic substances, activate the body's innate immunity and acquired immunity and induce the development

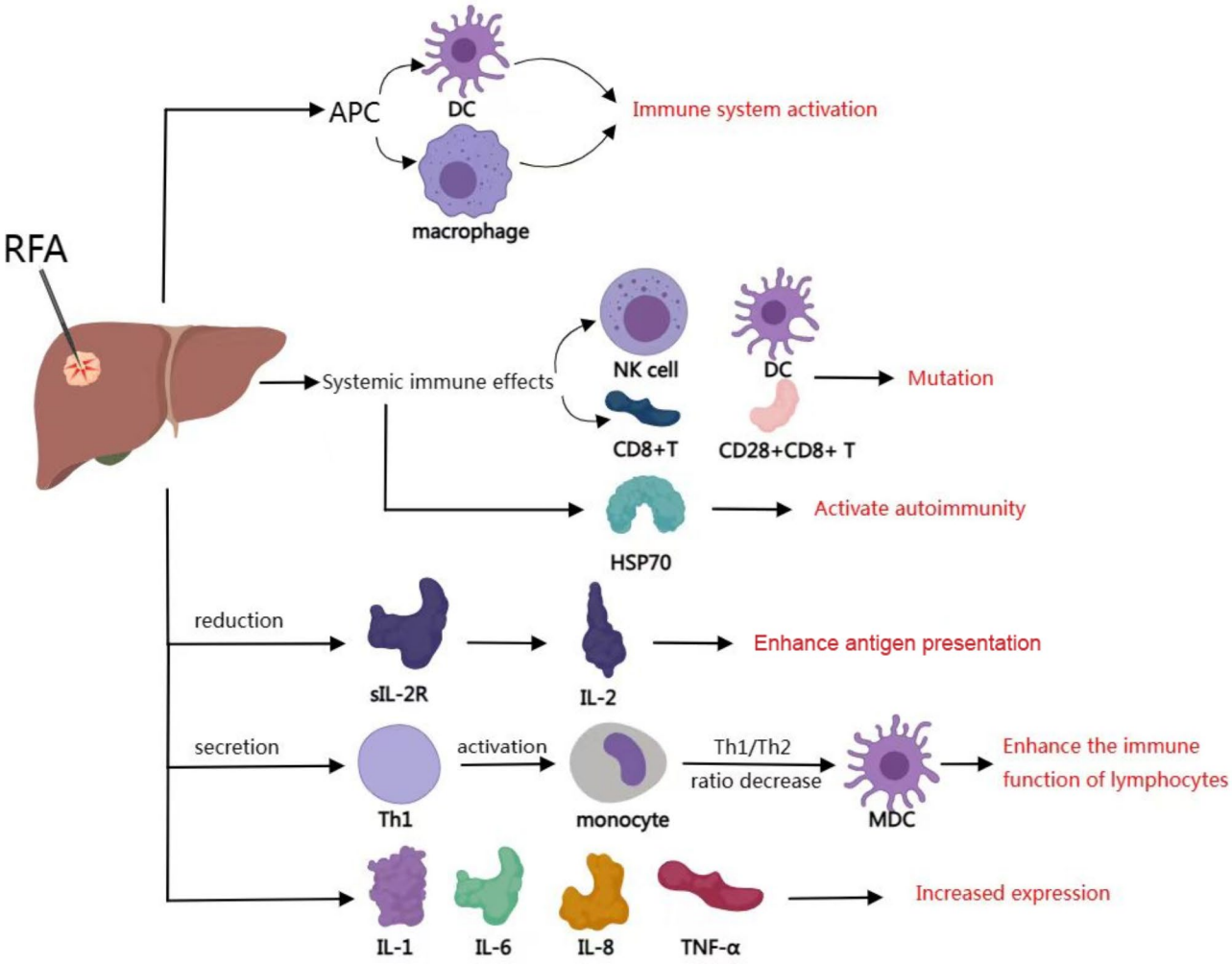


**Fig. 1** The anti-tumor immune mechanism of RFA

of specific immunity. Michael et al. found that RFA increased immunogenicity in HCC patients, thereby promoting endogenous adjuvant release and DC activation [44]. Zhang et al. reported that the level of IL-12 in peripheral serum increased significantly after one month of microwave ablation treatment in HCC patients, while the levels of IL-4 and IL-10 decreased significantly [45]. Experimental studies have shown that after RFA treatment of liver cancer tissue and normal liver tissue, the expression of heat shock protein at the edge of the coagulation area of cancer tissue is upregulated more and lasts longer. Heat shock protein, as a class of heat stress proteins, can activate and induce body-specific immune responses [46]. Ficogen-3 (FCN3) is significantly elevated after RFA treatment in HCC patients, while survival analysis revealed that patients with elevated Ficolin-3 have a better survival prognosis: the 1-, 2- and 3-year disease-free survival rates of patients with elevated Ficolin-3 were 73.3%, 60.0% and 50.0%, respectively, while those with no change in Ficolin-3 were 59.1%, 31.8% and 22.7%, respectively [47]. Another study from Zheng et al. revealed that expression of Ficolin-3 was down-regulated in HCC tissues, while over-expression of Ficolin-3 inhibits tumor growth in vivo. Furthermore, anti-C6 blocking antibody targeting Ficolin-3-mediated membrane attack complex formation rescued the inhibition of HCC growth caused by Ficolin-3 [48]. These data suggest that RFA and active immunotherapy with Ficolin-3 may have a synergistic effect in HCC treatment [47]. (2) Regulatory effect of immune cells: studies have unveiled that inflammatory cells mainly infiltrate in the transition zone of high-temperature ablation, including macrophages, DCs, NK cells, B cells, and T cells with specific anti-tumor ability [10, 45, 49–51]. Several studies have demonstrated that the number of CD3+, CD4 + T cells and the ratio of CD4+/CD8 + T cells is significantly increased after HCC thermal ablation [10, 45, 49]. For example, RFA induced CD8 + T cells, memory CD8 + T cells and DC, and down-regulated Treg in HCC mice. RFA also increased PD-1/PD-L1 expression, which was inhibited by sunitinib

and activated the immune response [51]. Mizukoshi et al. observed a significant increase in tumor-specific T cells after RFA in 62% of patients, suggesting that RFA causes tumor antigen release. Moreover, there is a significant correlation between tumor-specific T cells and recurrence-free survival [52]. RFA can regulate the Th1/Th2 ratio by stimulating antigen presentation, inhibiting soluble interleukin-2 receptor and transforming growth factor-β secretion, so that the Th1/Th2 ratio will increase and enhance the antitumor effect [45, 51]. Th17 is a newly discovered subpopulation of CD4 + T cells that play an important role in the immune response and disease progression in patients with chronic hepatitis virus B infection. It has been shown that microwave ablation can induce a transitional immune response by boosting the number of Th17 cells [49].

According to previous research, it is currently believed that the anti-tumor immune mechanism of RFA may involve: (1) reducing the tumor burden, so that the body's immunosuppressive state is relieved [46]; (2) exposing tumor cell surface antigen determinants or tumor antigen changes, thereby enhancing tumor antigenicity [10, 49, 50]. For example, necrotic tumor tissue as an allogeneic tissue becomes neoantigen, which stimulates the immune response and enhances the anti-tumor ability of the body after RFA [10, 46]; (3) in situ inactivation of the heat shock protein and further activation and inducing of the body's specific anti-tumor immune response [10, 46, 47]; (4) blockage of the secretion of certain cytokines and repair of the body's anti-tumor immune response to a certain extent [53]; (5) thermal ablation, which plays an important role in tumor angiogenesis inhibition, cancer cell apoptosis, antigen immunogenicity upregulation, reduction in immune cell apoptosis, or indirect promotion of immune cell differentiation [46, 53].

#### Ectopic effect of RFA in HCC therapy

Clinical studies have found that RFA can not only kill tumor cells but also induce the spontaneous regression of distant metastases in some HCC patients, resulting in an "ectopic effect". This may be caused by a variety of immunomodulatory effects of RFA of stimulating the body's specific anti-tumor immunity and enhancing the body's anti-tumor immunity while fighting against tumor immunosuppression [28, 47, 49, 53–55]. Compared with RFA or α-PD-1 mAb alone, RFA plus α-PD-1 mAb combination therapy significantly increased the proportion of TILs and maintained high levels of IFN-γ + or TNF-α + CD8 + or CD4 + T cells and the expression of IFN-γ and TNF-α in tumor tissues [56]. The above data suggests that RFA induces the body's anti-tumor immune response but is inhibited by active immunosuppression in the TME. However, RFA combined with PD-1 inhibitor therapy can enhance the RFA-induced anti-tumor immune response by blocking the PD-L1/PD-1 pathway and reverse immunosuppression at distant tumor sites. Other studies have also shown that T cells from distant non-RFA tumors exhibit a powerful and transient antitumor effect, but this weakens as the tumor grows back [57]. The ectopic effects observed in RFA treatment for HCC have also been validated in the treatment of other types of tumors. For example, radiation therapy has been shown to induce similar anti-tumor effects by triggering a specific immune response to tumor antigens. Radiotherapy upregulates the number of functionally active tumor-specific effector cells in the TME and, combined with the blocking of immune checkpoints, can improve the efficacy of radiotherapy for local and distant tumors [58]. The reduction in tumor burden level after ablative radiotherapy depends largely on the T cell response. Ablative radiotherapy significantly increases T cell initiation in drained lymphoid tissue, thereby eradicating the primary tumor or reducing distant metastasis in a CD8 + T cell-dependent manner [59]. Moreover, in one study, local immunotherapy could enhance the immune response induced by ablative radiotherapy, and the importance of immune activation in preventing tumor recurrence was highlighted [60]. These findings suggest that while reducing the tumor burden, we should also pay attention to the effect of anti-tumor immune enhancement. However, as for RFA, the factors that trigger innate and adaptive immunity remain elusive, and the underlying mechanisms deserve further exploration. In addition, how to prolong this anti-tumor immune effect is to be addressed and the feasibility of RFA combined with immunotherapy still needs to be further demonstrated by phase II and III large-scale clinical trials.

#### Anti-tumor effect of RFA combined with immunotherapy

Using mouse animal models, the rationality of RFA combined with an immunotherapy regimen has been verified. One study used a liver cancer model to test the combined effect of RFA and TLR9 stimulation and demonstrated that this combination prevents tumor spread [61]. Similar results were obtained for mouse B16 ovalbumin tumor models [62]. Anti-PD-1 antibody combined with RFA successfully overcame immune tolerance and induced the activation of systemic immune response in mouse colon cancer liver metastasis models [63]. RFA itself is able to trigger a strong T cell-mediated immune response in tumors. However, tumor cells inhibit CD8 + and CD4 + T cell function by upregulating the ratio of regulatory T cells to effector T cells and increasing PD-1/PD-L1 expression, resulting in immune escape. Nonetheless, after RFA in combination with PD-1 inhibitors, the ratio of regulatory T cells to effector T cells is reversed, resulting in significant tumor volume reduction and a significant prolongation of survival. This confirms

**Table 1** Research progress in RFA combined immunotherapy

| First author name et al. | Year | Research method | Control arm | Study type | Result |
|---|---|---|---|---|---|
| Kuang M et al. [73] | 2005 | MTC + CytoMPS | MTC + PBS | Animal experiments | The treatment method of MTC + CytoMPS can significantly improve the survival rate of tumor patients |
| Liu Q et al. [81] | 2009 | RFA + DC vaccination | RFA + PBS | Animal experiments | Receiving DC vaccination before RFA can enhance antigen-specific T cell responses and reduce tumor recurrence. |
| Chen Z et al. [74] | 2009 | MWA + GM-CSF + CTLA-4 | MWA + BSA + IgG | Animal experiments | Microwave ablation combined with injection of GM-CSF and CTLA-4 into the tumor can locally eradicate the tumor, stimulate tumor rejection, and treat distant metastasis of the tumor. |
| Iida N et al. [64] | 2010 | RFA + ECI301 | RFA + Blank | Animal experiments | ECI301 enhances RFA induced anti-tumor immune response in a CCR1 dependent manner. |
| Han X et al. [82] | 2019 | RFA/HIFU + PLGA-R837 | Blank | Animal experiments | RFA/HIFU causes PLGA to lyse and release R837, which can induce DC maturation, form specific anti-tumor immunity, and help clarify metastatic tumors. |
| Wang X et al. [65] | 2021 | RFA + Camrelizumab | RFA | PSM analysis | Combination therapy with anti-PD-1 plus RFA was superior to RFA alone in improving survival in patients with recurrent HCC. |
| Ma H et al. [66] | 2010 | RFA + Adoptive immunotherapy | Single-arm | Phase II clinical trial | RAK cell adoptive immunotherapy might be helpful in preventing recurrence in HCC patients after RFA. |
| Yu MA et al. [79] | 2015 | MWA + MDC + DC-CIK | MWA | open-label phase II study | Immunotherapy can improve the immune status and liver function of liver cancer patients. |
| Sawada Y et al. [72] | 2016 | RFA + GPC3 | Single-arm | Phase II clinical trial | Glycican-3 (GPC3) peptide vaccine can reduce the tumor recurrence rate in patients with GPC3 marker positive tumors. |
| Duffy AG et al. [55] | 2016 | RFA + Tremelimumab | TACE/CA + Tremelimumab | Clinical Trials.gov: NCT01853618; Registration Date: 2013-05-02; Clinical Trial Registry: National Cancer Institute. | Tremelimumab can enhance the activation effect of RFA on the immune system. |

MTC: microwave tumour coagulation; CytoMPS: intra-tumoural injectons of IL-2 and GM-CSF microparticles; RFA: Radiofrequency ablation; DC: dendritic cell; Microwave ablation: Microwave ablation; GM-CSF: granulocyte-macrophage colony stimulating factor; CTLA-4: cytotoxic T-lymphocyte-associated antigen 4; ECI301: an active variant of CC chemokine ligand 3; PEI: percutaneous ethanol injection; PSM: propensity score matching; CIK: cytokine-induced killer; MDC: myeloid dendritic cells; DC-CIK: dendritic cell- cytokine-induced killer; GPC3: glypican-3; HIFU: high intensity focused ultrasound; PLGA-R837: poly(lactic-co-glycolic) acid to en capsulate either R837 (Imiquimod)

that RFA combined with ICIs may be an effective treatment strategy.

At the same time, the safety of the RFA combined with immunotherapy towards advanced HCC is also well-guaranteed with a low risk of adverse events. In Iida et al.'s study, no adverse events, including hepatic failure or autoimmune responses, were detected after mice were treated with ECI301 and RFA against HCC [64]. Meanwhile, in another clinical trial including 127 patients with HCC [65], 41 patients were treated with RFA combined with anti-PD-1, and only 4 patients were detected with adverse events including pleural effusion (1 patient) and body temperature increase (3 patients), while 7 patients were diagnosed with the above adverse events in the control group (86 patients received RFA only).

## Advances in the combination of RFA and immunotherapy for HCC

Several studies on RFA were reviewed, as shown in Table 1 [65, 66]. RFA induces local tissue coagulation necrosis and results in the in-situ release of large amounts of cellular debris, which can serve as a source of tumor antigens to elicit an adaptive host immune response against the tumor [20]. The efficacy of immune checkpoint blockade therapy has been shown to be strongly correlated with pre-existing tumor antigen-specific T cell immune responses [67]. Several studies utilizing preclinical animal models have revealed that RFA can induce systemic T cell-mediated anti-tumor immunity [17, 68]. Antigen-specific T cell immune responses have also been observed in HCC patients treated with RFA [51, 69]. However, RFA-induced immune responses are often weak and thus unable to stop tumor progression and prevent tumor recurrence; the underlying mechanisms also remain ambiguous [10, 46, 55]. Furthermore,

these studies were limited to the analysis of peripheral immune cells rather than the TME. In addition to immunosuppressive pathways, various cytokines used to enhance T cell homeostasis and immune responses also enhance the anti-tumor efficacy of RFA [64, 70]. Therefore, combination therapy involving RFA and various immunomodulators can be explored clinically. RFA combined with immunotherapy can significantly increase the immune response after tumor ablation, create an enhanced immune environment in the body and around the ablation lesion, and consequently control the residual tumor, its recurrence and metastasis.

To date, immunotherapies targeting PD-1 and its ligand PD-L1 have achieved good and lasting efficacy in a variety of tumors. PD-L1 is expressed in tumor cells and certain immune cells, which inhibits T cell immunity by binding to PD-1 on the surface of T cells and makes tumor cells able to escape T cell attack. PD-1/PD-L1 monoclonal antibody was used for the targeted blocking of the PD-1/PD-L1 signaling pathway, which could restore the anti-tumor activity of T cells and induce the apoptosis of tumor cells, while PD-1/PD-L1 monoclonal antibody treatment alone could not significantly improve the prognosis of liver cancer patients. In a mouse model of CRLM, it was found that RFA treatment not only increased T cell infiltration but also upregulated PD-L1 expression. The combination of RFA and PD-1 monoclonal antibody could significantly enhance T cell immune response, upregulate the number of effector T cells, enhance anti-tumor immunity, and prolong survival. Clinically, compared with patients (BCLC B stage) using CTLA-4 inhibitor (tremelimumab) combined with TACE, Duffy et al. used RFA combined with tremelimumab in the treatment of patients with advanced liver cancer (BCLC C stage) and demonstrated the potential value of RFA combined with tremelimumab as a new treatment option. PR was achieved in 26% of patients in the combination group, with 6- and 12-month progression-free survival rates of 57.1% and 33.1%, respectively. Moreover, the median tumor progression time was 7.4 months (95% CI: 4.7–19.4 months) and the median OS was 12.3 months (95% CI: 9.3–15.4 months). The combination of RFA and tremelimumab significantly downregulated Treg cells and increased CD8 + T cell infiltration in the tumor microenvironment. What is more, the viral load was significantly reduced in 12 of the 14 HCV patients, and the median viral load decreased from $1275 \times 10^3$ IU/mL to $351 \times 10^3$ IU/mL [55].

#### RFA combined with immunomodulator therapy

RFA combined with immunomodulators can promote stronger anti-tumor immunity in the body, resulting in improved efficacy [64, 71–74]. Studies have shown that the microwave ablation of liver cancer combined with intratumoral injection of IL-2 or granulocyte macrophage colony-stimulating factor can significantly enhance the anti-tumor immune response and improve prognosis compared with microwave ablation or cytokine injection alone [73, 74]. In a phase II clinical trial of RFA combined with glypican-3 peptide vaccine for the treatment of advanced liver cancer, Sawada et al. found that patients who received GRC3 peptide vaccine after RFA had significantly lower 1- and 2-year recurrence rates than those who received RFA alone [72]. Iida et al. reported that the macrophage inflammatory protein 1α variant, an immunomodulator, could significantly amplify the specific anti-tumor immune response induced by RFA in a mouse liver cancer model. The increased infiltration of immune effector cells, such as CDllc+, CD4 + and CD8+, was found in both ablated and non-ablated tumor sites [64].

#### RFA combined with adoptive immunotherapy

The use of cytokine-induced killer (CIK) cells is a novel immune cell therapy for a variety of malignant tumors, owing to the strong anti-tumor activity of T lymphocytes and the advantages of major histocompatibility complex restriction tumor killing of NK cells [28, 75, 76]. Several single-center randomized controlled trials have analyzed the effect of CIK-assisted surgery in the treatment of HCC and reported that CIK can improve the disease-free survival and OS of HCC patients with good safety. For example, Weng et al. randomly divided 85 HCC patients into two groups, administering either TACE combined with RFA and adjuvant CIK therapy (CIK adjuvant therapy group, $n = 45$) or no CIK adjuvant therapy (no CIK adjuvant group, $n = 40$). The tumor recurrence rates at 1 year and 18 months in the CIK adjuvant treatment group were 8.9% and 15.6%, respectively, and they were 30% and 40%, respectively, in the no CIK adjuvant group [77]. Zhao et al. followed up HCC patients who received CIK combined with RFA after TACE, and the relapse rates were lower in the combination group than in the control group (12% vs. 25.8%). At the same time, most patients showed reduced HBV DNA content after combination therapy [78]. Another study showed that CIK or DC-CIK sequential TACE combined with RFA treatment can not only directly act on tumors but also remove residual HCC cells in the body and improve the overall immune function of patients after surgery, prolong survival, and slow down recurrence, presenting a good prospect of combination therapy for HCC [75]. One clinical study also found that the combination of RFA and RAK cells effectively reduced the risk of HCC recurrence: none of the 7 HCC patients had recurrence at 7 months of follow-up. After the combination of RFA and RAK, the proportion of CD3+/CD8 + cells and the concentration of peripheral blood IFN-γ continued to increase, while the proportion of CD4+/CD8 + decreased

[66]. Previous studies have reported that DC in combination with MWA can improve anti-tumor immune cell subsets in HCC patients [80]. Research has shown that RFA increases DC infiltration and induces immune activation, but this immune response is not strong enough to inhibit metastatic tumor growth [81]. Moreover, DC combined with RFA eliminated more than 90% of recurrent tumors in HCC mice [82]. Han et al. used an oil-water emulsification method to synthesize PLGA-R837 nanoparticles with a diameter of about 100 nm by wrapping R837 with PLGA, and in vitro experiments confirmed that this significantly promoted DC maturation. The use of these nanoparticles in mouse subcutaneous tumor models could enhance the production of TAA in the primary tumor. At the same time, radiofrequency heat caused PLGA to lyse and release R837, inducing DC maturation, and mature DC presenting TAA to T cells to form specific anti-tumor cell immunity, thereby clearing metastatic tumors [83]. Given that RFA, PLGA and R837 have now been approved by the FDA, the combined RFA-immune activation strategy mediated by these nanoparticles has the potential of providing further options for clinical translation and application.

#### RFA combined with local immunotherapy

Recently, RFA combined with immunotherapy has made definite progress in HCC treatment. Given the unique immunosuppressive microenvironment and immunogenicity of HCC, however, only a small number of HCC patients have shown an objective tumor response with long-term survival benefits, so this treatment model needs to be further explored. In addition, systemic immunotherapy may lead to severe autoimmune toxicity in patients, and this risk increases dramatically with the combination of anti-PD-1/PD-L1 and anti-CTLA-4. Intralesional immunoinjection is a new therapeutic strategy that aims to use tumors as their own vaccines [84]. When the drug is injected directly into the tumor, higher drug concentrations can be obtained locally with lower drug doses. For example, iodine ($^{131}$I)-labeled metuximab ($^{131}$I metuximab) has been shown to benefit HCC patients after RFA [85]. RFA combined with local immunoinjection can prevent systemic exposure and the off-target toxicity of drugs. Furthermore, because RFA can appropriately activate the circulation of anti-tumor immune cells, this stimulation can generate systemic anti-tumor effects while enhancing the initiation of local tumor-specific immunity. Intratumoral immunotherapy enables the biopsy of tissue samples at each injection, which can better reflect the dynamics of local immunity. It is expected that through more clinical trials, the efficacy and safety of RFA combined with local immunotherapy for HCC can be evaluated more accurately.

#### Pseudo-progression after immunotherapy and RFA

Pseudo-progression was firstly described in melanoma patients in 2008 and widely reported thereafter in many other malignancies including HCC, non-small cell lung cancer, metastasis center nervous system cancer, etc. It is a phenomenon representing as the increase in tumor size after the application of immunotherapy and explained as the infiltration of immune cells among the lesion inducing the edema and necrosis of tumor tissue. From our perspective of clinical practice, pseudo-progression is also detected in patients receiving immunotherapy. However, among those receiving immunotherapy after RFA, to our knowledge, no case of pseudo-progression has been observed or reported by other doctors. We infer that this is because the process of RFA had ablated the tumor tissue thoroughly, leaving no place for immune cells to infiltrate, not to mention the edema or necrosis of lesions.

#### Disadvantages of combination therapy

On the one hand, as both RFA and immunotherapy have certain requirements of liver function in patients, combined treatment has the risk of inducing further deterioration of poor liver function or even liver failure. On the other hand, performing sequential therapy of RFA and immunotherapy can be a significant financial burden, especially at present, as the price of immunotherapy is still high, although there are many related reimbursement and cost-reduction policies in China.

In addition, the disadvantages of this combination therapy are mainly due to the individual shortcomings of RFA and immunotherapy. For example, the usage of RFA is usually limited by the location of the lesions. Specifically, when the tumor is located too close to major intrahepatic blood vessels, the heat generated by RFA for tumor ablation is partially dissipated by the blood flow, which inevitably reduces the efficacy of tumor ablation as well as the immune response stimulated by RFA [2]. As for the immunotherapy, it takes effect via a specific immune checkpoint, which means that a portion of patients cannot benefit from the expected curative effects even after bearing a certain financial burden [2]. Furthermore, patients undergoing immunotherapies may also suffer from the adverse effects. For instance, treatment with PD-1/PD-L1 inhibitors may lead to a high risk of anemia (45.4%), followed by fatigue (34.3%), dysphagia (30.0%), as reported by Zhou et al. [86] For patients receiving nivolumab alone, rash was observed in 15–30% of them, while this portion was elevated to 30-45% when nivolumab was used in combination with ipilimumab [87].

## Discussion

RFA is one of the key treatment options for HCC, which can not only be used as a radical treatment for early HCC, bridging therapy before liver transplantation and salvage liver transplantation [88], but also as a debulking and symptom-reducing measure for intermediate and advanced liver cancer [89]. RFA yields an OS and local recurrence rate similar to surgery, while it brings the advantages of less trauma, fewer complications, high safety, and reproducibility. However, due to the presence of microsatellite lesions that cannot be accurately detected before and during surgery, the recurrence rate of HCC after RFA alone is particularly high and its management remains a significant clinical challenge [90, 91]. Abnormalities in the body's immune microenvironment are closely related to the occurrence and development of cancers, including HCC. Immunotherapy is a promising treatment option for some advanced or metastatic cancers. The occurrence, development, treatment and prognosis of HCC are closely related to the tumor immune microenvironment and immune function. Immunotherapy has also shown new advantages in the systemic treatment of liver cancer, while the limited immune response rate restricts its further development. RFA can induce the body to produce tumor-specific antigens, promote the proliferation of immune cells and improve the immunosuppressive microenvironment; it has the effects of activating the body's immune system and enhancing antitumor immune function to a certain extent. Theoretically, the release of tumor antigens after RFA will recruit CTLs to microsatellite lesions, while appropriate immunotherapy can inhibit microsatellite lesions and intrahepatic metastasis [92]. Some preclinical studies and a small number of clinical studies have shown that RFA can enhance the anti-tumor immune response, whereas this is insufficient to delay the growth of distant tumors. At the same time, ablation combined with immunotherapy can further strengthen the anti-tumor response, delay distant tumor growth, and reduce tumor recurrence and metastasis, providing new ideas for HCC systematic treatment [55].

The application of RFA combined with immunotherapy appears to have a good synergistic effect. This approach in HCC has good efficacy and controllable safety, with the formulation of individualized combination therapy being a current research focus. However, insufficient RFA (iRFA) is also a problem that cannot be ignored and is the main cause of recurrence after RFA. It may cause HCCs to have a more aggressive phenotype and worse prognosis; it promotes residual tumor proliferation, migration, invasion, stemness, angiogenesis, drug resistance, and epithelial-mesenchymal transformation through epigenetic and transcriptional regulation [93–100]. Some combination therapy strategies can mitigate the iRFA-induced more aggressive phenotype and worse prognosis in HCC, which incorporate the administration of metformin, hydroxychloroquine, sorafenib, bevacizumab, CTLA-4 inhibitors, or IFN-α. These data suggest that the adverse effects of iRFA can be addressed by systemic therapy or immunotherapy. RFA alone can enhance the body's anti-tumor immunity while fighting tumor immunosuppression, enhancing its value and frequency of application in clinical practice. In combination with immunotherapy, RFA can also significantly improve the body's anti-tumor immunity, delay the time of tumor recurrence and metastasis, thus has broad application value and can provide new options for the treatment of advanced HCC. However, the regulatory mechanism of RFA on immune function and anti-tumor immunity is not completely understood, and the selection and use of immunotherapy and its combination with other adjuvant therapies still need in-depth research to explore the optimal treatment plan. To this end, it should be noted that the need for individualized treatment of HCC patients cannot be ignored in the study of RFA combined with immunotherapy. Ablation efficiency is affected by various factors such as tumor size, tumor location, ablation equipment, and the duration and frequency of ablation. Meanwhile, immunotherapy efficiency is also influenced by multiple factors, such as the form of administration and drug dose. Apparently, the factors influencing RFA combined with immunotherapy are complex, including the optimal timing of immunotherapy, the choice of adjuvant immunotherapy, and the order of application of RFA and immunotherapy. Important future research directions of HCC treatment may include how to better combine RFA with immunotherapy and other adjuvant therapies and how to reduce the tumor burden more effectively after effective ablation, so that the body can produce more effective anti-tumor immunity, and reduce and delay tumor recurrence and metastasis. In addition, the efficacy of this combination treatment regimen still needs to be verified by multi-center, large-scale clinical trials, which should consider the following main directions: (1) screening of HCC patients suitable for combination therapy; (2) determination of the drug selection, dosage application standards and drug cycle of a specific combination drug; (3) development of evaluation criteria for efficacy and search for effective markers that can predict the disease development trend.

## Conclusion

On the basis of the reviewed literature, we have reason to believe that RFA combined with immunotherapy is a highly potent strategy to reduce the tumor burden of HCC patients, slow down systemic tumor cell metastasis, improve their quality of life, and prolong long-term survival.

**Abbreviations**

| | |
|---|---|
| CIK | Cytokine-induced killer |
| CRLM | Colorectal cancer metastasis |
| CTLA-4 | Cytotoxic T lymphocyte associated antigen |
| CTLA-4 | Cytotoxic T lymphocyte associated antigen |
| DC | Dendritic cell |
| HCC | Hepatocellular carcinoma |
| HSP-70 | Heatshockprotein-70 |
| IL | Interleukin |
| iRFA | Insufficient RFA |
| NK | Natural killer |
| RFA | Radiofrequency ablation |
| PD-1 | Programmed cell death receptor-1 |
| PD-L1 | Programmed cell death receptor-ligand 1 |
| PLC | Primary liver cancer |
| TACE | Transarterial chemoembolization |
| TAA | Tumor-associated antigen |
| TIL | Tumor infiltrating lymphocyte |
| TME | Tumor microenvironment |
| TNF-α | Tumor necrosis factor-α |

**Author contributions**

Research idea: GLX, TWY and ZQX. Data extraction and integrated analysis: GLX and ZHZ. Quality assessment and result interpretation: ZHZ, Modifcation and polishing: GLX, TWY and ZQX. Manuscript writing: All authors. Final approval of manuscript: All authors.

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**Data availability**

Contact the correspondence authors if necessary.

## Declarations

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no competing interests.

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## Publisher's note