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Mechanisms and therapeutic strategies to combat the recurrence and progression of hepatocellular carcinoma after thermal ablation

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ABSTRACT

Thermal ablation (TA), including radiofrequency ablation (RFA) and microwave ablation (MWA), has become the main treatment for early-stage hepatocellular carcinoma (HCC) due to advantages such as safety and minimal invasiveness. However, HCC is prone to local recurrence, with more aggressive malignancies after TA closely related to TA-induced changes in epithelial-mesenchymal transition (EMT) and remodeling of the tumor microenvironment (TME). According to many studies, various components of the TME undergo complex changes after TA, such as the recruitment of innate and adaptive immune cells, the release of tumor-associated antigens (TAAs) and various cytokines, the formation of a hypoxic microenvironment, and tumor angiogenesis. Changes in the TME after TA can partly enhance the anti-tumor immune response; however, this response is weak to kill the tumor completely. Certain components of the TME can induce an immunosuppressive microenvironment through complex interactions, leading to tumor recurrence and progression. How the TME is remodeled after TA and the mechanism by which the TME promotes HCC recurrence and progression are unclear. Thus, in this review, we focused on these issues to highlight potentially effective strategies for reducing and preventing the recurrence and progression of HCC after TA.

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide, with a 5-year survival rate of approximately 18%.[1] As a result, HCC treatment remains a major global health challenge. Thermal ablation (TA) is a well-known radical treatment for early-stage HCC. Compared to hepatectomy and liver transplantation, TA is associated with minimal invasion, safety, and rapid recovery. For patients with a small single HCC, TA has similar efficacy to surgical resection.[2] However, recent clinical data suggest that HCC recurs in a few patients after TA, and malignant transformation (e.g., sarcomatoid transformation[3]) may occur, causing significant enhancements in proliferation, invasion, and metastatic potential.[4,5] Therefore, the potential mechanism of HCC progression after TA has been gradually explored.

According to recent studies, the main macroscopic cause of local HCC recurrence after TA is incomplete TA of the primary HCC.[6] Incomplete TA can lead to residual tumor recurrence in different ways, as described below: (1) The temperature of the ablation zone exhibits a gradient distribution and can be divided into three zones (Fig. 1): the core ablation zone higher than 60 °C, in which the tumor suffers from coagulation necrosis[7,8]; the transition zone between 43 °C and 50 °C, in which tumor cells are subjected to sublethal heat stress (SHS) and prone to residual disease and tumor recurrence[9]; and the surrounding liver tissue at normal temperature. (2) A significant heat sink effect exists during radiofrequency ablation (RFA) of HCC around large blood vessels, resulting in a local sublethal temperature that cannot completely kill the tumor.[10] A study revealed that the presence of vessels at least 3 mm in size adjacent to hepatic tumors is an independent predictor of incomplete radiofrequency ablation (IRFA).[11] (3) Tumor cells attached to the ablation needles may metastasize along the track.[12]

At the microscopic level, in addition to malignant transformation of the biological behavior of residual HCC cells (e.g., epithelial-mesenchymal transition [EMT][13] and enhancement of stemness[14,15]), TA causes remodeling of the tumor microenvironment (TME) and its

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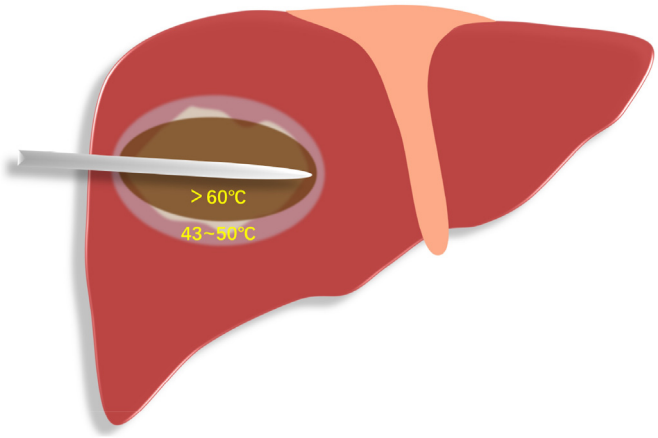

Fig. 1. Temperature gradient distribution in the ablation zone.

interaction with residual HCC cells.[16] In this review, we aimed to summarize how the TME is remodeled after TA and the mechanisms by which TA promotes HCC recurrence and progression. In addition, we highlighted the therapeutic strategies expected to be translated into clinical practice.

2. Remodeling of the HCC microenvironment after TA and relevant mechanisms in the promotion of tumor recurrence and progression

2.1. Thermal ablation and tumor microenvironment

The TME is the environment in which tumors develop and comprises cellular and non-cellular components. In addition to tumor cells, cellular components of the HCC microenvironment include immune cells, hepatic stellate cells (HSCs), and endothelial cells (ECs). Non-cellular components include the extracellular matrix (ECM), cytokines, blood vessels, and lymphatic networks. Studies have shown that TA induces systemic anti-tumor immunity by destroying tumor cells and causing the release of a variety of immunogenic substances.[17-19] Anti-tumor cells, such as dendritic cells (DCs) and natural killer (NK) cells, can be observed in the ablation transition zone,[20,21] suggesting that TA promotes anti-tumor immune responses. Pre-clinical and clinical studies have reported tumor recurrence and malignant enhancement after ablation.[5,22] Zang et al. showed that the anti-tumor immune response induced by ablation is transient in patients with HCC.[23] In mice, Wang et al. reported that the increased apoptosis of distant tumor cells in the local RFA group will recover to the level in the non-RFA group after a short time.[24] Qi et al. reported that lysates from RFA-treated tumors significantly increase programmed cell death protein 1 (PD-1) expression in tumor-infiltrating $CD4^+$ and $CD8^+$ T cells by enhancing hepatocyte growth factor (HGF) expression.[25] Altogether, heat stress induces complex alterations in the immune status of local and distant TME, inducing weak anti-tumor immune responses and pro-tumor immune responses. Various immunosuppressive cells (e.g., M2 macrophages) and negatively regulated cytokines (e.g., interleukin 6 (IL-6) and transforming growth factor-beta (TGF-β)) in the TME promote the formation of an immunosuppressive microenvironment and tumor progression after TA,[26] as shown in Fig. 2.

2.2. Roles of various cellular components in HCC progression after TA

2.2.1. Myeloid-derived suppressor cells (MDSCs)

MDSCs are immature myeloid cells of monocytic and granulocytic lineages released from the bone marrow. MDSCs in the TME play an important role in HCC recurrence and progression after TA. Although RFA can enhance the specific immune response of T cells to a variety of tumor-associated antigens (TAAs), T cells are inhibited by MDSCs.[27] Furthermore, the memory phenotype and life span of T cells are insufficient to completely prevent HCC recurrence. Mechanically, heat-mediated upregulation of METTL1 enhances TGF-β2 translation and induces an increase in $CD11b^+CD15^+$ PMN-MDSCs to suppress $CD8^+$ T cells.[28] The elimination of PMN-MDSCs by blocking the METTL1-TGF-β2-PMN-MDSC axis significantly alleviates IRFA-induced tumor progression and restores the $CD8^+$ T cell population. Palliative RFA (pRFA) accelerates the progression of residual tumors by increasing the number of MDSCs and reducing the T cell-mediated anti-tumor immune response.[29] According to the researchers, the enhanced infiltration

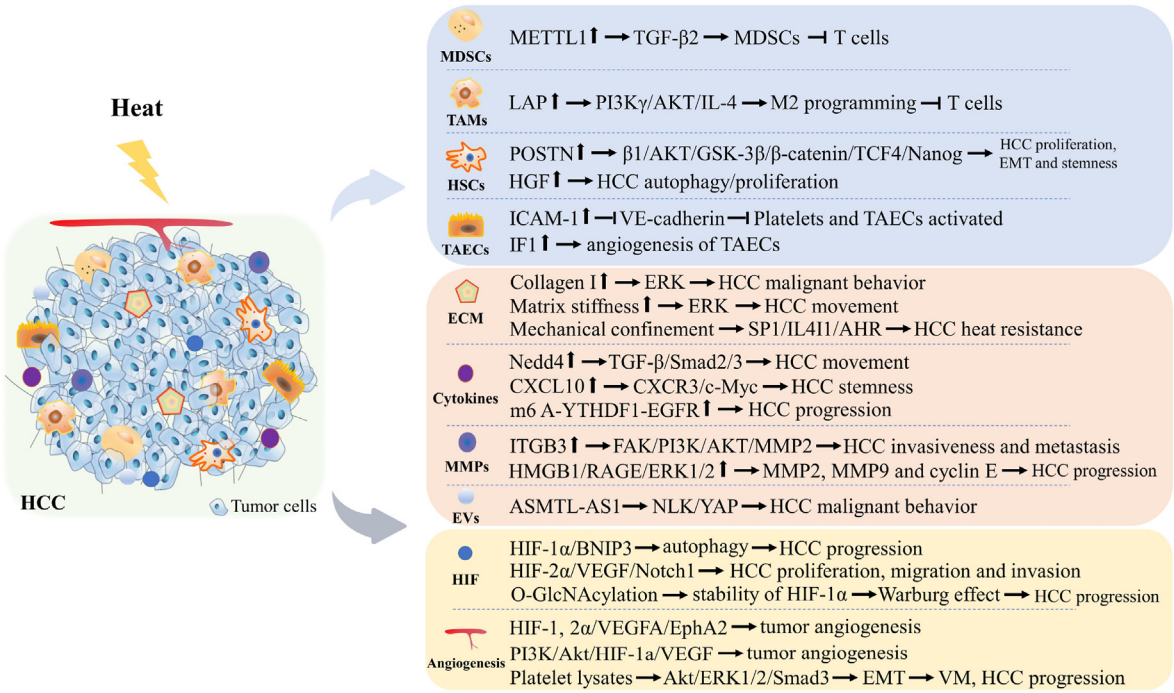

Fig. 2. Mechanisms of HCC recurrence and progression after TA.

of MDSCs may be due to the release of pro-inflammatory factors after heat stress. MDSC depletion delays tumor progression after pRFA, which may be related to the reversal of immunosuppression. The above studies mainly focused on the suppressive effect of MDSCs on T cells after TA; however, MDSCs may have an even more impact on the TME. For example, MDSCs can block antigen presentation by transferring oxidized lipids to DCs and inhibit the innate immune function of NK cells via NKp30 receptors.[30,31] In addition, MDSC can recruit regulatory T cells (Tregs) through the production of CCR5 ligands or interact with macrophages through the production of IL-10.[32,33] These functions of MDSCs may exist in the TME after TA; however, such evidence is lacking. To eliminate the immunosuppression of MDSCs, studies have verified that blocking the recruitment of MDSCs, promoting their differentiation, and inhibiting their metabolism can contribute to anti-tumor efficacy.[34-37] Therefore, targeting MDSCs may be a therapeutic strategy to inhibit HCC recurrence after TA.

2.2.2. Tumor-associated macrophages (TAMs)

TAMs can undergo M1 and M2 polarization, in which M1 macrophages play an anti-tumor role while M2 macrophages promote tumors.[38] Studies have shown that many macrophages cluster in the transition zone,[39,40] suggesting that TAMs are involved in the recurrence and progression of residual HCC after TA. Mechanistically, Liu et al. revealed that macrophages phagocytize heat-treated cells through LC3-associated phagocytosis (LAP), activate the PI3Kγ/AKT pathway, and promote IL-4-mediated M2 programming of macrophages, resulting in the inhibition of T cell proliferation by expressing anti-inflammatory cytokines.[41] These researchers identified that macrophages receiving LAP are a major source of CCL2 and CCL7, which recruit more macrophages to promote the progression of residual tumors. Targeting PI3Kγ reprograms infiltrating macrophages and promotes anti-PD-1-mediated tumor regression. As one of the main components of immunosuppression, the role of TAMs in HCC recurrence after TA has not been fully explored because TAMs boost a variety of tumors through complex mechanisms. For instance, TAMs can inhibit $CD8^+$ T cell function through direct contact or the secretion of immunosuppressive cytokines (such as TGF-β). In addition, the metabolism and polarization of TAMs can be affected by Tregs,[42] indicating crosstalk between immunosuppressive cells. To block the immunosuppression of TAMs, studies have shown that tumor progression can be prevented by inhibiting the production of M2 TAMs, reprogramming M2 TAMs to the M1 phenotype, and blocking the communication between M2 TAMs and other cells in TME.[43-45] However, therapeutic strategies targeting TMAs remain to be explored to improve the prognosis of patients with residual HCC after TA.

2.2.3. HSCs

HSCs are mesenchymal cells unique to the liver and are located in the lumen of the disc surrounding the hepatic sinusoids. The ECM in the liver is mainly secreted by human stem cells (HSCs). Studies have shown that activated HSCs can significantly promote the proliferation, EMT, and stemness of tumor cells and reduce their apoptosis when co-cultured with heat-exposed HCC.[46,47] Regarding the mechanism, periostin (POSTN) in conditioned media from activated HSCs (HSC-CM) activates the integrin β1/AKT/GSK-3β/β-catenin/TCF4/Nanog axis; however, the inhibition of POSTN by calcipotriol can block this effect. In addition, when heat-treated HCC cells are co-cultured with HSC-CM, HSC-derived HGF promotes a change in the biological state of tumor cells from autophagy to proliferation.[48] HGF regulates autophagy via HGF/c-Met signaling and ATG5/Beclin1, or proliferation via downstream components of cyclinD1. Based on these studies, HSCs promote the progression of residual HCC through various secretions. A prior study revealed that different subsets of HSCs have different functions in hepatocarcinogenesis.[49] Quiescent HSCs can produce HGF to prevent HCC development. In contrast, activated HSCs promote tumor proliferation by secreting type I collagen to increase liver stiffness and activate the discoid domain-containing receptor 1. Their proposal to restore the balance between different subsets of HSCs may be of great value in treating HCC recurrence after TA.

2.2.4. Cancer-associated fibroblasts (CAFs)

CAFs are activated fibroblasts in the tumor stroma and are non-neoplastic mesenchymal cells. Kumar et al. revealed that increased α-SMA-positive activated myofibroblasts exist at the ablation margin after RFA, and daily treatment with celecoxib after RFA reduces this recruitment.[40] Rozenblum et al. indicated that many activated myofibroblasts accumulate in the ablation boundary area after RFA and promote collagen deposition in mice, with potential knockout of myofibroblast accumulation via IL-6 knockout.[50] Although the accumulation of CAFs after TA is observed in the above studies, the mechanisms of CAFs are not involved in residual HCC recurrence. As an important component of the TME, CAFs play a complex role in tumor progression.[51] On one hand, CAFs induce immune cells to differentiate into cancer-promoting subtypes, enhance the activity of immunosuppressive cells, and inhibit immune effector cells by secreting various soluble factors. On the other hand, CAFs impede anti-tumor immune cells by remodeling the ECM. Recent studies have shown that immunotherapies that deplete CAFs and inhibit their function exhibit powerful anti-tumor effects.[52,53] However, the roles of CAFs in tumor progression after TA and therapeutic strategies remain to be explored.

2.2.5. Tumor endothelial cells (TECs)

The inner layer of tumor blood vessels is formed by TECs; however, normal vascular barrier function is lost and permeability is increased, which is closely related to tumor metastasis. A previous study revealed that the upregulation of ICAM-1 in TECs after IRFA leads to the downregulation of VE-cadherin, which in turn activates platelets and promotes the permeability of TECs; this effect can be reversed by platelet depletion or ICAM-1 inhibition.[54] IF1 (ATPase inhibitory factor 1) in HCC cells directly and indirectly affects the angiogenesis of TECs after IRFA, and HCC cells can induce vasculogenic mimicry (VM) in the presence of TECs.[55] The above studies indicate that TECs promote residual HCC progression through crosstalk with different components in TME. TECs can promote angiogenesis through fructose and ceramide metabolism in HCC.[56,57] According to another study, ECs boost immunosuppressive macrophages through oncofetal reprogramming in HCC.[58] Overall, these studies suggest that the role of TECs in HCC recurrence after TA needs further investigation, and targeting the metabolism of TECs may serve as one of the preventive strategies.

2.3. Roles of different non-cellular components in HCC progression after ablation

2.3.1. ECM

Different cells within the TME, such as CAFs and ECs, produce ECM, the main components of which are collagen, lamin, hyaluronic acid, and proteoglycan.[59] A large amount of collagen deposition occurs around the ablation zone, which promotes ECM remodeling.[50] Zhang et al. demonstrated that heat treatment induces a significant accumulation of type I collagen at the edge of the ablation zone and promotes the malignant behavior of residual HCC cells by activating the ERK pathway.[60] Their subsequent study was performed using cell culture gels with different stiffnesses to explore the effect of matrix stiffness change on residual HCC after TA.[61] Based on their results, the increase in matrix stiffness contributes to the movement and progression of residual HCC cells, in which the activation of the ERK pathway plays an important role. In addition, a recent study showed that mechanical confinement contributes to the heat resistance of HCC via the SP1/IL4I1/AHR axis.[62] In brief, matrix remodeling can be sensed by residual HCC cells to achieve recurrence. In tumors, the ECM participates in immune cell regulation through mechanical properties and soluble factors.[63] Previous studies have suggested that ECM components may induce a DC phenotype with low immunogenicity to interfere with tumor antigen presentation and

anti-tumor immunity.[64] In addition, certain components of the ECM, such as laminin 511, can impede the activation of effector T cells.[65] The spatial barrier formed by the high matrix density of the ECM restricts T cells from recognizing cancer cells.[66] Therefore, the complex connection between the ECM and the immune system greatly impacts the progression of tumors, which may play a role in HCC progression after TA. Targeting ECM components (such as hyaluronidase) or removing fibroblasts has been demonstrated to suppress tumor progression.[67,68]

2.3.2. Cytokines

Cytokines, secreted by various cells, are a class of small-molecule soluble proteins that play regulatory roles between cells. Heat stress is reported to increase the synthesis and secretion of various cytokines, including TGF-β,[29] HGF,[69] IL-6,[70] CCL2,[71] etc. METTL14-mediated N6-methyladenosine modification induces the upregulation of Nedd4, which directly binds to the TGF-β type I receptor and forms K27-linked ubiquitin at lysine 391, thereby enhancing the TGF-β pathway and ultimately promoting residual HCC progression.[72] C-X-C motif chemokine 10 (CXCL10) upregulates $CD133^{+}$ CSCs by activating the CXC receptor 3/c-Myc pathway, which may accelerate HCC recurrence after RFA.[73] Su et al. revealed that the enhancement of epidermal factor growth receptor (EGFR) expression by SHS is achieved by increasing the modification of EGFR m6 A near the 5′ untranslated region and its binding to YTHDF1. At the same time, silencing of YTHDF1 and inhibition of EGFR synergistically suppress the malignant transformation of HCC cells.[74] Dai et al. reported that heat exposure significantly increases EGFR phosphorylation without affecting the total expression of EGFR.[75] AG1478, an EGFR-specific inhibitor, suppresses the proliferation of HCC cells, confirming the important role of EGFR transactivation. Another *in vitro* study revealed that heat stress induces rapid autophosphorylation of EGFR in the absence of growth factor ligands; however, inhibition of EGFR has little effect on enhancing heat stress-induced HCC cell killing,[76] which contradicts the findings of Dai et al. Cytokines play crucial roles in the interactions between cellular components as key mediators of cell communication in the TME. For instance, IL-6 and stromal cell-derived factor 1a secreted from CAFs can induce MDSC activation.[77] TGF-β promotes HCC progression by inducing the polarization of Tregs.[78] As cytokine dysregulation is present at all stages of tumor evolution,[79] cytokine engineering (such as the formation of "supercytokines" and "immunocytokines"[80]) may be a powerful approach in the inhibition of HCC recurrence after TA.

2.3.3. Extracellular vesicles (EVs)

EVs are membranous vesicle structures released by donor cells and contain various components, such as proteins, lipids, nucleic acids, and metabolites,[81] which act on recipient cells and regulate their functions. Ma et al. revealed that the lncRNA, ASMTL-AS1, is transactivated by MYC after IRFA and promotes the expression of NLK by secluding miR-342-3p, finally activating YAP signaling in HCC.[82] Exosome-encapsulated ASMTL-AS1 transmits malignancy between residual HCC cells through the NLK/YAP pathway. Therefore, EVs are crucial communication tools for cell-to-cell dialogues in the TME. In HCC, hexokinase 1 in HSCs undergoes palmitoylation upon TGF-β stimulation and secretion from EVs, which the tumor hijacks to accelerate glycolysis and progression.[83] Hence, EVs are expected to serve as therapeutic targets, and different cell sources have been employed for EV production in clinical trials. For instance, loading gemcitabine into autologous pancreatic cancer-derived exosomes for chemotherapy significantly inhibits tumor growth.[84] However, the role of EVs in the heat-induced recurrence and progression of HCC remains unclear, and treatment strategies are yet to be developed.

2.3.4. Matrix metalloproteinases (MMPs)

MMPs in the ECM are a family of zinc-dependent endopeptidases that can degrade collagen and participate in signal transduction and immune regulation.[85] Degradation of collagen plays an important role in the process of tumor invasion and metastasis. Several studies have reported that the expression levels of MMP2 and MMP9 are upregulated after TA.[70,86] IRFA has been demonstrated to induce the upregulation of integrin 3 and mediate the increase in MMP2 expression by activating the FAK/PI3K/AKT pathway, thereby enhancing the metastatic ability of HCC cells.[87] HMGB1 from dead tumor cells after IRFA activates the ERK1/2 pathway by binding to the receptor for advanced glycation end product and upregulates the expression of MMP2, MMP9, and cyclin E1, ultimately promoting the proliferation and migration of residual HCC.[88] MMPs play an important immunomodulatory role in tumor promotion. For example, macrophages expressing MMP11 promote breast cancer cell migration and monocyte recruitment through CCL2-CCR2 signaling.[89] SB-3CT, an MMP2/MMP9 inhibitor, significantly downregulates PD-L1 expression and enhances the anti-tumor effect of anti-CTLA-4.[90] These studies suggest that the blockade of MMPs is a potential strategy to inhibit the invasion and metastasis of residual HCC after TA.

2.4. Hypoxia

Hypoxia promotes the survival, proliferation, invasion, metastasis, angiogenesis, and EMT of the tumor by inducing the upregulation of hypoxia-inducible factor (HIF) expression.[91,92] IRFA can lead to blood stagnation and thrombosis in the transition zone,[93] resulting in the formation of a hypoxic microenvironment, an increase in HIF-1α,[94] and the promotion of the recurrence and progression of residual HCC. By simulating the survival environment of residual cells after IRFA via heating and hypoxic culture, Tong et al. reported that HIF-1α depends on TGF-β to activate downstream pathways to facilitate the survival and EMT of MHCC97H and SMMC7721 stem cells.[93] In addition to HIF-1α, TA can induce the upregulation of HIF-2α expression,[95] and promote the proliferation, migration, and invasion of HCC cells through the HIF-2α/VEGF/Notch1 axis,[96] or promote angiogenesis through the HIF-1/-2α/VEGFA/EphA2 axis.[97] Xu et al. showed that heat stress promotes the progression of HCC cells by enhancing autophagy through the HIF-1α/BNIP3 pathway *in vitro*,[98] suggesting an interaction between hypoxia and autophagy.

2.5. Metabolic reprogramming

Metabolic reprogramming is considered a hallmark of cancer and is closely related to the hypoxic microenvironment, which refers to the proliferation of tumor cells by changing their metabolic mode to adapt to stressful conditions (e.g., hypoxia and nutrient deficiency). According to previous studies, HIF is closely related to glucose metabolism in tumors.[99] Our recent study identified that heat treatment enhances O-GlcNAcylation, which improves the stability of HIF-1α, thereby enhancing the Warburg effect and promoting the EMT and progression of residual HCC.[100] The suppression of O-GlcNAcylation hinders residual tumor progression, suggesting the therapeutic potential of targeting metabolic reprogramming. In addition to glucose metabolism, metabolic reprogramming is involved in fat and amino acid metabolism; however, their roles in HCC recurrence after ablation remain unknown.

2.6. Tumor angiogenesis

During the evolution of solid tumors, the formation of new tumor blood vessels is induced to obtain oxygen and nutrition.[101] VEGF, fibroblast growth factor (FGF), and HGF are the driving factors of tumor angiogenesis in HCC,[102] among which VEGF is the most comprehensively studied. Several studies have shown that the expression levels of VEGF and VEGF receptor (VEGFR) are upregulated in residual HCC after SHS, promoting abnormal vascular proliferation and accelerating HCC recurrence and progression.[103,104] IRFA increases the expression of VEGF through the CaMKII/ERK pathway to promote the proliferation of HCC cells.[105] The RFA-mediated HGF/c-Met pathway and VEGF activation in the normal liver can promote distant subcutaneous tumor growth.[106] IRFA can promote angiogenesis by inducing the stemness of human HCC

tissues. At the same time, amarogentin inhibits tumor angiogenesis by affecting the p53-dependent VEGFA/Dll4/Notch1 pathway.[107] Tan et al. showed that VEGFR2 is downregulated after IRFA, and inhibition of VEGFR2 cannot block the migration and stemness of HCC cells.[108] In contrast, VEGFR1 expression is upregulated, and blocking VEGFR1 can alleviate the progression of HCC, suggesting that VEGFR1 is a potential therapeutic target. Kong et al. revealed that high temperatures exert a stronger pro-angiogenic effect by enhancing the PI3K/Akt/HIF-1α/VEGFA pathway,[109] suggesting that hypoxia promotes angiogenesis. In addition to angiogenesis, residual tumors can induce VM for survival. Jia et al. suggest that RFA-induced platelet lysates may boost tumor metastasis and VM via EMT mediated by Akt/ERK1/2/Smad3 signaling,[110] suggesting that residual tumors survive through different angiogenic patterns. Thus, combination treatment comprising different processes is a potential strategy.

3. Strategies to prevent and treat HCC recurrence after TA

3.1. Improving the rate of pathological complete ablation

The main cause of HCC recurrence and progression after TA is incomplete ablation, which is related to the presence of microvascular invasion in the peritumoral tissue, satellite lesions, and the size of the ablation margin. Studies have shown that patients with local tumor progression have a smaller minimal ablation margin,[111] and the expansion of ablation margins can significantly reduce the risk of tumor recurrence and improve the long-term survival rate of patients,[112,113] suggesting that ablation margins play a key role in complete pathological ablation. Therefore, the pathological complete ablation rate should be improved by expanding the ablation margin, and the peritumoral microvascular invasion and micrometastases should be eliminated.[114] In a prior study, 96 patients (a total of 188 liver cancer nodules with a median diameter of 2.5 cm) were treated with stereotactic RFA, which resulted in a complete pathological response rate of 97.3% in the liver tissue and 96.2% in 52 patients with nodules ≥3 cm in diameter.[115] Xu et al. showed that intraoperative CT/ultrasound fusion imaging technology can achieve more satisfactory results when applied to the TA of HCC nodules in inconspicuous, high-risk sites or sizes >3 cm.[116] In a clinical study, Joo et al. demonstrated that enhanced CT during microwave ablation (MWA) could reveal the potential suboptimal minimal ablation margin and immediately guide additional ablation to obtain an adequate ablation margin, thereby improving the efficacy.[117] The progress of image-guided technology, three-dimensional navigation technology, and image fusion technology and their combination with RFA helps improve the complete ablation rate of tumors, thereby improving the prognosis of patients.

3.2. Evaluating the risk of tumor recurrence and monitoring early recurrence after TA

After ablation, assessing the risk of tumor recurrence and monitoring early recurrence are positive preventive strategies that facilitate further management as early as possible in patients. Many biological markers can be used to evaluate tumor recurrence after ablation, including serum markers (e.g., VEGF,[118] sCTLA-4,[119] and CXCL10[73]) and molecular markers in surgical specimens (e.g., keratin 19[120]). However, these markers remain to be translated into clinical guidelines. In addition to markers, early tumor recurrence can be predicted using imaging techniques such as CT texture analysis,[121] multi-parametric MRI,[122,123] and ultrasound.[124] Kobe et al. showed that the difference in normalized peak enhancement and hepatic artery perfusion (ablation zone tumor) obtained from the fusion of preoperative MR and posttreatment CT perfusion images can be used to predict local tumor recurrence after RFA.[125]

3.3. TA plus targeted therapy or immunotherapy

As mentioned above, TA releases numerous TAAs that mediate the anti-tumor immune response[126]; however, the immune response is insufficient to eliminate residual tumors. In addition, residual tumors induce the formation of an immunosuppressive microenvironment. Therefore, enhancing the anti-tumor immune response and reducing immunosuppression based on local TA is a reasonable strategy. Combining TA with targeted therapy or immunotherapy is expected to prevent the recurrence and progression of residual tumors. In the past decade, sorafenib and lenvatinib have been the first-line treatments for advanced liver cancer. A meta-analysis revealed that compared with that in TA alone, RFA or MWA combined with sorafenib had better efficacy in the treatment of HCC; however, the incidence of adverse reactions (e.g., hand-foot skin reaction and gastrointestinal reaction) was significant,[127] suggesting that the safety of this combination therapy needs to be evaluated. Studies have shown that treatment with sorafenib inhibits the viability, invasion, metastasis, and EMT of HCC cells after heat exposure.[128] In addition, Zhang et al. showed that sorafenib blocks the interaction between the ECM protein collagen I and residual HCC cells by disrupting ERK signaling, thereby preventing tumor progression.[60] However, heat-induced hypoxia enhances the tolerance of HCC cells to sorafenib.[93] IRFA-induced upregulation of IF1 in tumor cells attenuates the effect of sorafenib by activating the NF-κB pathway.[55] In addition to sorafenib, sunitinib synergistically enhances anti-tumor immunity. Qi et al. showed that sunitinib enhances the anti-tumor immune response by inhibiting the upregulation of PD-1 in tumor-infiltrating T cells induced by the HGF pathway, reducing the proportion of Tregs, and inhibiting the expression of PD-L1 in DCs induced by VEGF.[25] Although TA combined with targeted therapy can significantly inhibit residual HCC, tumor resistance is still challenging.

Many scholars have combined TA with immunotherapy to obtain and enhance systemic anti-tumor immune responses. In recent years, immunotherapies, such as immune checkpoint inhibitors (ICIs), have led to encouraging results in patients with advanced HCC. PD-1 and CTLA-4 are the main inhibitory immune checkpoint molecules, among which the interaction between PD-1 and its ligand, PD-L1, inhibits the activation of T cells, whereas CTLA-4 on T cells can inhibit effective antigen presentation and enhance the immunosuppressive function of immune Tregs.[129] Studies have shown that combining TA and ICIs induces significant residual tumor suppression. Tumor growth and angiogenesis after TA can be inhibited by bevacizumab.[109] Through a clinical study, Duffy et al. showed that the combination of teremumab and ablation leads to the accumulation of $CD8^+$ T cells in tumors; however, the relative contribution of ablation to efficacy requires further study.[130] Zhang et al. showed that, compared with that in RFA or anti-CTLA-4 treatment alone, combination therapy led to a lower subcutaneous tumor growth rate, longer survival time, and higher $CD4^+$ lymphocyte expression and IFN-γ production in mice.[131] These results indicate that anti-CTLA-4 can inhibit residual tumors. Using a mouse rectal liver metastasis tumor model, Shi et al. demonstrated that the combination of RFA and anti-PD-1 can significantly enhance the immune response of tumor antigen-specific T cells, increase the ratio of effector T cells to Tregs in distant tumors, and inhibit the growth of distant tumors.[132] However, their follow-up study revealed that the inflammation caused by RFA blocks the anti-tumor efficacy of PD-1 inhibitors.[71] In addition, RFA, combined with the immunomodulator Resiquimod (R848), can stimulate a stronger anti-tumor immune response, inhibit angiogenesis, promote cell apoptosis, and effectively inhibit the progression of HCC in mice in an NK cell-dependent manner.[133] Adoptive cellular therapy (ACT) has demonstrated great potential in immunotherapy tumors. ACT refers to extracting T cells from patients, culture, and modification *in vitro*, followed by reinfusion into the patient to destroy tumor cells.[134] Zhong et al. used MWA to destroy tumors on one side of bilateral tumor-bearing mice and intravenously injected dendritic cell-derived exosomes (Dex) or dendritic cells to achieve combined treatment.[135] Their results showed

that, compared with that in MWA alone, combination treatment significantly inhibited tumor growth, increased the number of $CD8^+$ T cells at the tumor site, and decreased the number of Tregs, indicating the potential of TA plus ACT.

Great progress has been made in eliminating residual tumor recurrence using multi-drug combinations. Co-delivery of the MDSCs inhibitor, IPI549, and αPD-L1 antibody using nanotechnology can effectively solve the tumor recurrence problem by blocking the compensatory increase in PD-L1 expression on MDSCs caused by IRFA combined with MDSC inhibition.[136] Moreover, the combination of OK-432 and αPD-1 antibodies significantly stimulates DCs and promotes $CD8^+$ T cell infiltration and function in residual tumors.[137] Through clinical trials, Kitahara et al. showed that patients treated with RFA and OK432 have longer recurrence-free survival than those treated with RFA plus basic-protocol DCs.[138] A clinical study revealed that combining RFA and sequential cellular immunotherapy improves progression-free survival in patients with HCC.[139] Clinical trials of multi-drug combinations are ongoing (e.g., NCT04727307 and NCT05277675).[140]

3.4. TA plus other treatments

Aspirin inhibits the proliferation, invasion, and metastasis of residual VX2 tumors through an anti-inflammatory effect.[141] RFA and the antimalarials, chloroquine (CQ)/hydroxychloroquine (HCQ), inhibit the autophagy, proliferation, and stemness of tumor cells and promote their apoptosis.[142-144] This effect can be enhanced by combining CQ and c-Met inhibitors.[48] Metformin reduces the proliferation, migration, and invasion of HCC cells,[145] and the expression of stem cell markers.[46] All-trans retinoic acid (ATRA) eliminates tumor-initiating cells through the PI3K/AKT pathway.[146] The combination of IFN-α and Songyou Yin (SYY) reduces EMT and lung metastasis in HCC after IRFA.[147] Arsenic trioxide (ATO) inhibits tumor angiogenesis by blocking the paracrine signal of Ang-1 and Ang-2 via inhibition of p-Akt/HIF-1α.[148] RFA combined with brachytherapy (e.g., iodine-125) can control the local recurrence of residual HCC.[149,150] Tan et al. combined percutaneous intratumoral ethanol injection (PEI) and RFA to achieve improved efficacy in two ways: PEI induces microthrombus formation and occlusion of blood vessels, thereby reducing heat loss; and combination therapy can inhibit the increase in Ki-67 and VEGF and decrease in caspase-3 to a certain extent.[151]

Nanomedicines show great potential for enhancing the effects of drugs or the immune system against heat-exposed HCC. Arsenic-loaded zeolite imidazole frame-8 nanoparticles (As@ZIF-8 NPs) lead to more significant inhibition of the growth and metastasis of residual HCC than by ATO.[103] D-mannose-chelated iron oxide nanoparticles (man-IONPs) prepared by Cui et al. can transform the immunosuppressive microenvironment into an immunoactivating microenvironment (i.e., polarize M2 macrophages into the M1 phenotype), thereby inhibiting the progression of residual tumors after insufficient MWA.[152] Nanoparticles of mannose-derived carbon dots (Man-CDs) developed by our team can effectively take up multiple DSs (e.g., DAMPs and multiple histones) after MWA and improve the presentation of DS and tumor antigens to promote the maturation of DCs and enhance the specific immune response.[153] Furthermore, our team prepared another nanomedicine that can capture and present antigens to promote the maturation of tumor-infiltrating DCs by upregulating the m6A modification level through the intracellular release of loaded FTO inhibitors.[154] However, the clinical transformation of nanomaterials faces many challenges, such as a lack of data on the long-term effect of nanomaterial-based immunotherapy on TME and the difficulty of predicting the response of tumor heterogeneity to nanodrugs.[155] Further research is required in this area. The combination therapies with TA are shown in Table 1.

Table 1
Combination therapies and their mechanisms of action.

Treatment in combination with TA	Mechanism of action	Reference
Sorafenib	Blockade of the interaction of collagen I and HCC Inhibition of HCC malignant behavior	60,128
Sunitinib	Inhibition of Tregs, PD-1 in T cells and PD-L1 in DCs	25
Bevacizumab	Inhibition of tumor growth and angiogenesis	109
Teremumab	Accumulation of $CD8^+$ T cells	130
CTLA-4 blockade	Higher $CD4^+$ lymphocyte expression and IFN-γ production	131
PD-1 blockade	Enhancement of tumor antigen-specific T cell response; the increase in intratumoral Teff to Treg ratio	132
Resiquimod	The increase in functional NK cells; inhibition of angiogenesis; the promotion of tumor cells apoptosis	133
Dex	The increase of $CD8^+$ T cells and the decrease of Tregs	135
IPI549+αPD-L1 antibody	Blockade of PD-L1 expression on MDSCs	136
OK-432+αPD-1 antibody	Enhancement of $CD8^+$ T cells infiltration and function	137
Aspirin	Inhibition of HCC malignant behavior	141
CQ/HCQ	Inhibition of autophagy, proliferation and stemness of tumor; the promotion of tumor cells apoptosis	142-144
Metformin	Inhibition of HCC malignant behavior	145
ATRA	Suppression of TICs by inhibiting PI3K/AKT pathway; the promotion of tumor cells apoptosis	146
Songyou Yin	Inhibition of HCCcells EMT and lung metastasis	147
Arsenic trioxide	Inhibition of p-Akt/HIF-1α to block the paracrine of Ang-1 and Ang-2 to suppress angiogenesis	148
Iodine-125	The creation of a radiated area leading to tumor necrosis	149,150
PEI	Forming microthrombus, blocking blood vessels and reducing heat loss; inhibition of Ki-67 and VEGF; decrease of Caspase-3	151
As@ZIF-8 NPs	Inhibition of HCC growth and metastasis	103
Man-IONPs	Transformation of M2 macrophages into M1	152
Man-CDs	Promotion of the maturation of DCs by improving the presentation of DS and tumor antigens	153
Man/mal-MPDA@FB23-2	Maturation of TIDCs promoted by m6A modification enhanced by loaded FTO inhibitors	154

4. Summary and prospect

TA is an important method for the treatment of early-stage HCC and has the advantages of minimal invasiveness and safety. However, preclinical and clinical studies have shown that the immune response induced by TA is weak to destroy all tumor cells. In addition, residual tumor cells subjected to SHS undergo metabolic reprogramming and immune escape by promoting the formation of an immunosuppressive microenvironment, which leads to a high tumor recurrence rate after TA. The remodeling of TME after TA promotes tumor recurrence and progression via complex mechanisms. Understanding these mechanisms is essential for unleashing the full potential of the immune system to improve patient outcomes. As previously mentioned, the blockade of immunosuppressive cells (e.g., MDSCs and TAMs) and inhibition of negatively regulated cytokines (e.g., VEGF and EGFR) have exhibited significant efficacy. Moreover, the complexity of the microenvironment requires combined strategies. Thus, a promising treatment strategy involves enhancing the immunogenic effects of ablation therapy and eliminating immunosuppression by combining targeted therapy and immunotherapy. However, studies on certain treatment strategies are still lacking. For example, blocking CAF-derived signals (e.g., chemokines)[156] and switching the tumor-promoting CAF subtype to a quiescent phenotype[157] can achieve clinical benefits. However, the application of these strategies for HCC recurrence after TA has not been reported. In

addition, studies have shown that exosomes can be used as vaccines or specific drug delivery vehicles for cancer immunotherapy.[158,159] If TA is combined with exosome-based immunotherapy, it may be more conducive to enhancing anti-tumor immunity. Further studies are required to explore potential therapeutic strategies to improve survival rates and reduce tumor recurrence and metastasis. In current clinical practice, the efficacy of certain combination therapies is still dismal, which may be attributed to the complexity of the TME. TA combined with targeted immunotherapy may not completely eliminate residual tumors. To overcome this clinical problem, efforts are focused on developing multimodal treatments for HCC after TA. In addition, applying TME biomarkers to monitor the efficacy of combination therapy helps optimize treatment strategies.

Although studies on the relevant mechanisms and combination therapies have made significant progress, many limitations still exist. In terms of experimental models, most studies have used non-cirrhotic tumor models, which cannot highly simulate the liver environment of human HCC. In addition, the heterogeneity of HCC causes tumor cell subsets to differ in many aspects (e.g., growth rate, invasiveness, and drug sensitivity), posing great challenges to treatment. Certain studies only used a few HCC cell lines; therefore, tumor heterogeneity could not be simulated. Regarding simulating incomplete ablation, most experiments did not monitor the temperature change at the tumor site during the ablation process; however, the ablation temperature and duration in patient-derived xenograft mice were quite different from the clinical situation. Regarding clinical data, obtaining residual HCC tissue after ablation is difficult; therefore, pathological and molecular evidence from human HCC samples is lacking. Regarding combination therapy, studies on their side effects, such as whether a few drugs (such as small-molecule kinase inhibitors) have off-target effects, the impact of inhibitors on human organs, and potential systemic toxicity, are lacking. Solutions to these problems will enable scientific findings to be translated for use in the clinic.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

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