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REVIEW

An Overview of Hepatocellular Carcinoma After Insufficient Radiofrequency Ablation

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Abstract: Radiofrequency ablation (RFA) is a commonly used treatment for hepatocellular carcinoma (HCC), however, various complex conditions in clinical practice may lead to insufficient radiofrequency ablation (IRFA), allowing residual HCC to survive. In clinical practice and laboratory models, IRFA plays an important role in rapid tumor progression. Therefore, targeting the residual HCC and avoiding IRFA were worthwhile methods. A deeper understanding of IRFA is required; IRFA contributes to the improvement of proliferative activity, migration rates, and invasive capacity, and this may be due to the involvement of multiple complex processes or proteins, including epithelial mesenchymal transitions (EMTs), cancer stem cells (CSCs), autophagy, heat shock proteins (HSPs), changes of non-tumor cells and extracellular matrix, altered immune microenvironment, hypoxia-inducible factors (HIFs), growth factors, epigenetic alterations, and metabolic reprogramming. We focus on the processes of the above mechanisms and possible therapeutic approach, with a review of the literature. Additionally, we recapitulated the construction methods of various experimental models of IRFA (in vivo and in vitro).

Keywords: hepatocellular carcinoma, insufficient radiofrequency ablation, epithelial mesenchymal transitions, residual viable tumors

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and the fifth most common cancer in the world.[1,2] Patients with early-stage HCC are usually treated curatively by hepatectomy or radiofrequency ablation (RFA). RFA, a minimally invasive approach, inducing coagulative necrosis of tumor tissue through an electrode placed in the tumor, offers better safety, fewer complications and, shorter hospital stay compared to hepatectomy.[3–6] Some studies have shown that RFA is inferior to surgical resection in terms of the higher risk of recurrence and metastasis,[7–10] and RFA resulted in 5-year overall recurrence rates of 63.5%, much higher than that of 41.7% in patients underwent surgical resection.[11] Several early clinical studies found rapid tumor progression in some patients after RFA, which may be due to residual viable tumors after RFA.[12–17] It has been found that thermal ablation damage is divided into three regions, namely the central high-temperature zone, the sublethal temperature transition zone, and the surrounding normal tissue. In the transition zone, tumors suffer reversible damage and eventually survive, thus leading to rapid tumor progression in a activate state.[18,19] The mechanism is complicated after IRFA and may include the following aspects: 1) IRFA causes Epithelial Mesenchymal Transitions (EMT), which is an important cause of tumor metastasis;[20–36] 2) IRFA leads to autophagic survival and plays a role in subsequent progression and metastasis;[37–44] 3) IRFA causes a significant increase in the number of cancer stem cells;[21,24,31,37,45–47] 4) IRFA triggers a hypoxic microenvironment that aids tumor cell survival and proliferation;[24,34,38,44,48–51] 5) IRFA causes sustained local inflammation with predominant myeloid suppressor cells, which inhibits the function of T cells in tumors;[52] 6) Heat shock response aids tumor cell survival after IRFA;[21,25,39,43,47,53] 7) IRFA stimulates the expression of several growth factors and receptors;[24,36,42,46,48,49,54–57] 8) IRFA activates non-tumor cells such as hepatic stellate cells (HSCs), tumor-associated endothelial cells, and platelets to help tumor cell survival and metastasis;[10,27,30,42,45,58] 9) IRFA causes epigenetic

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changes;[26,31,32,34] 10) IRFA enhances metabolic reprogramming of HCC.[30,34] The above mechanisms do not exist independently, but contribute to each other and together increase the malignancy of residual viable tumors.

In this review, we first briefly describe the experimental models currently in use. Next, we describe how IRFA induces cell survival, proliferation, metastasis, and invasion through EMT, cancer stem cells, autophagy, heat shock response, immune microenvironment, and hypoxia-inducible factors. In addition, the coping strategies for IRFA are briefly discussed.

Experimental Modeling of Insufficient Radiofrequency Ablation

To construct as realistic a model of IRFA as possible, researchers have used several different methods to create suitable models. Obara et al heated HCC cells in water baths at different temperatures from 37 degrees to 55 degrees for ten minutes and first proposed sublethal temperatures for cell models of HCC cells between 47 degrees and 49 degrees.[59] Hence, 47 or 48 degrees eventually became the sublethal temperature of cells for the vast majority of studies. The duration of heat treatment varies between institutions, but a water bath for ten minutes is the simplest and most commonly used method. A considerable number of studies simulate IRFA through the gradient of heating time of 5min, 10min, 15min, 20min, and 25min. In addition to this, considering that RFA can lead to the formation of a transition zone between normal liver tissue and necrotic coagulation, blood stagnation, and thrombosis exposing residual tumor cells to a hypoxic microenvironment,[60] Tong et al performed incubation of cells in a hypoxic incubator ($1\%O_2$) after heat treatment at 47 degrees to simulate the hypoxic environment to which tumor cells are exposed after thermal ablation.[24] In general, compared to unheated HCC cells, HCC cells after sublethal heat treatment exhibit higher proliferative activity, higher migration rates, and greater invasive capacity. Microscopically, heated cells often exhibited a spindle cell-like morphology with less intercellular adhesion and loss of polarity.

In studies with animal models, BALB/c mice have become the most frequently used animals, and New Zealand rabbits have also been used in some studies. IRFA models are usually constructed by the following methods. In mice, for example, the cells after heat treatment in vitro were directly transplanted into the liver or subcutaneous, and the follow-up experiments were carried out after tumor formation. Some studies transplant untreated tumor cells into the liver or subcutaneous for subsequent ablation, while other studies first implant heat-treated cells or IRFA tumor blocks into the subcutis or liver of tumor-bearing mice, and then cut up the blocks for "replantation" after tumor formation, which may facilitate stable tumor growth. In vivo, To partially ablate the tumor, the power of the ablation needle will be lower than that used clinically, and a considerable number of studies chose to ablate for 30s with a power output of 5 watts. The temperature range of the incompletely ablated zone as seen by infrared imaging was 41 degrees to 50 degrees, with the temperature in the center of the ablation greater than 50 degrees and the surrounding normal tissue temperature less than 40 degrees. Similar to heated liver cancer cells, incompletely ablated tumor tissue often exhibits faster growth rates, larger final tumor volumes and tumor specimens show that IRFA can cause more tumor metastases than tumor tissue with no ablation. Supplementary Table S1 summarizes the model construction methods that have been used.

Epithelial Mesenchymal Transition

EMT is closely associated with embryonic development, organogenesis, repair of damage, and migration and invasion of malignant tumors.[61,62] The main manifestation is that tumor epithelial cells undergo phenotypic transformation after receiving some stimulation and turn into mesenchymal cells with migration ability. The main molecular markers are E-cadherin, which is down-regulated, and N-cadherin, vimentin, Snail, and Twist, which are up-regulated.[63,64] Dong et al conducted research related to EMT on SMMC7721 and Huh7 cells after IRFA for the first time in 2013, demonstrating that EMT markers were significantly increased in surviving cells after heat treatment. Other studies have confirmed the occurrence of heat-induced EMT.[20] Yoshida et al found that this process was reversible. Expression of EMT markers (Snail, TWIST1, CHD1L, and COL1A1) showed an upward trend 5 days after treatment at 50°C, and returned to baseline level on day 12 after IRFA. Su et al found that IRFA could lead to epigenetic alterations in tumor cells, which subsequently led to EMT, but the relevant indicators decreased gradually over two days, suggesting its reversibility and plasticity.[21] This heat-induced EMT is likely to occur within a short period, suggesting that drugs should be applied early after RFA to inhibit the activation of EMT.

P-ERK1/2 has an important role in heat-induced EMT. Four studies[20,21,23,28] performed heat treatment on MHCC97H, HepG2, HuH7, and HEP3B cell lines and found that ERK was significantly phosphorylated. EMT was attenuated after inhibition of P-ERK1/2, which was similar to other ways of activating EMT.[65–67] They found that P13K, P46-Shc, and Periostin were activated as upstream proteins of ERK after heat treatment, causing tumor invasion and metastasis. WNT pathway and FLOT1/FOLT2 were also found to be activated by heat induction, which together promotes nuclear translocation of β-catenin and causes EMT.[22,29] Other classical signaling pathways or proteins are also activated. Zhou et al found significantly elevated IL-6 expression in heat-treated H22 cells, which in turn activated Jak2 and stat3 thereby triggering EMT.[33] Consistent with previous studies, NF-κB also plays a role in heat-induced EMT.[30,68,69]

Epigenetics has been reported to have an important relationship with EMT.[70,71] Heat-induced stress can induce a significant rise in the "writer" METTL3 and m6A, meanwhile, two "readers" IGF2BP1 and YTHDF1 are elevated, which in turn induces significant elevations in CD47 and EGFR expression or translation, ultimately inducing the occurrence of EMT.[10,36] Non-coding RNAs also play a role in heat-induced EMT, and different lncRNAs may play opposite roles, with FUNDC2P4 being significantly downregulated in HCC tissue remaining after RFA,[26] in contrast to ASMTL-AS1 being significantly elevated.[32] The possible signaling pathways are listed in Figure 1. Notably, although ZEB1 has been reported to produce a role in EMT in HCC,[72] Zeb1 is not currently reported to be activated in heat-induced EMT in HCC.

Cancer Stem Cell

Cancer stem cells (CSC) are described as a small group of cancer cells that can self-renew and may be responsible for recurrence, metastasis, and drug resistance.[73,74] In HCC, the main markers of CSCs are EpCAM, CD90, CD44, CD133, CD24, CD13, Nanog, and OCT4.[75–77] Sub-lethal heat treatment or hypoxic environments can lead to the generation of tumor stem cells. Among studies involving IRFA and HCC, CD133,[21,37,45–47] CD90,[24] CD44,[46,47] EpCAM,[45,46]

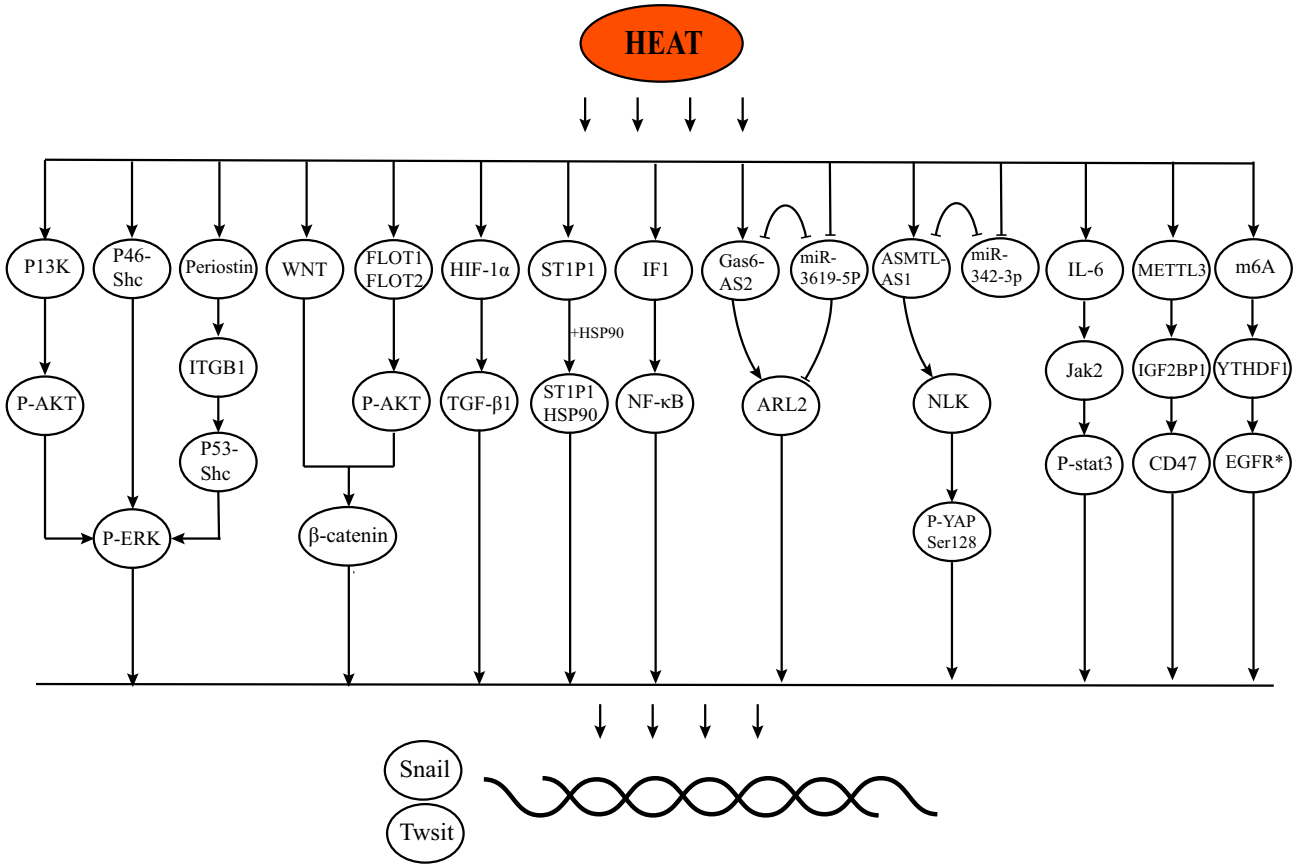

Figure 1 The possible signaling pathways of IRFA and EMT.

Nanog[31,45] and OCT4[31] are reported to be induced. Zhang et al reported similar expression trends of CD133 with CD31 and VEGFA in the IRFA-liver cancer tissue, suggesting that IRFA may affect tumor angiogenesis through CSCs,[57] and similar findings have been reported by other studies.[24,46] Interestingly, activated HSCs may promote the progression of residual tumor cells after thermal ablation by enriching CSCs.[45]

Autophagy

In the progression of cancer, autophagy plays a dual role of inhibition or promotion depending on the type and degree of tumor progression and genetic background.[78–83] Similarly, autophagy has been reported to play a paradoxical role in HCC. It protected cells from carcinogenesis at the early stage and promoted tumor progression at the advanced stage.[84,85] Previous studies have shown that sublethal heat stress induces autophagy in several human cancer cell lines, such as HeLa and A549 cells.[86] In this review, we summarize the effect of IRFA on autophagy in HCC. To our knowledge, Wang et al first reported that autophagy plays an important role in the elevated survival and invasiveness of Huh-7 and SMMC7721 cells after IRFA. When autophagy was inhibited (autophagy inhibitors such as 3-MA or chloroquine), the malignancy of the cells is reduced. Interestingly, this process is synchronized with the expression trend of CD133, suggesting a link between autophagy and CSC.[37] In addition, the hypoxic environment may also act synergistically with autophagy. Zhao et al observed an increase in the LC3B protein region consistent with a region of high HIF-1a expression in tumor tissues.[38] Xu et al knocked down the BNIP3 in heat-induced hepatocellular carcinoma cells and LC3B and HIF-1α showed a common downward trend.[41] Another study came to a similar conclusion.[44] As mentioned previously, HSCs are a non-negligible role in the survival and tumor progression of HCC cells. Zhang et al co-cultured surviving HCC cells after IRFA with HSCs culture medium (HSC-CM) and found that HSC-CM could increase the expression of LC3B at the early stage, suppress the cell apoptosis and promote the survival of cell autophagy. Expression of LC3B was gradually down-regulated and CyclinD1 was gradually up-regulated in the later stages.[42] This dynamic change demonstrates an important role for heat-induced autophagy and again suggests an important role for HCSs in this process. Table 1 shows the potential relations or mechanisms of autophagy and IRFA.

Heat Shock Proteins

When cells are under stress (such as extreme temperature, hypoxia, and drugs), some proteins undergo denaturation or folding.[87,88] Heat shock proteins (HSP) can reverse or inhibit this process, making HSP an important regulator of many important processes such as cell proliferation and differentiation.[89] Because protein misfolding occurs frequently in tumor cells, their survival and proliferation are more dependent on HSP than normal cells.[90,91] Previous studies have reported that HSP plays a key role in the occurrence and development of HCC.[92–94] We describe here the changes in HSP after IRFA. After the tumor cells were heated in a 50-degree water bath for 10 min, Yoshida et al found that HSP 27, 70, and 90 increased significantly on the fifth day after heat treatment and returned to the baseline level after 12 days.[21] Similarly, Zaimoku et al reported that the induction of HSP70 promoter increased with the increase of culture temperature or heating time in HepG2 and Huh7.[47] Another study demonstrated elevated heat-induced expression of HSP.[39] Two studies reported that heat induction can cause increased expression of stat3, which binds to HSP90 as a complex to promote survival. HSP90 inhibitors can reduce the complex formation and promote apoptosis in tumor cells.[25,53] Two studies by Chen et al found that the HSP90/Akt/mTOR pathway is involved in the signaling between autophagy and HSPs, and subsequent studies found that the HSP90 inhibitor 17-AAG, in combination with the autophagy inhibitor 3-mA, promoted apoptosis more significantly than monotherapy, suggesting an association between heat-induced heat shock processes and autophagy.[39,43] HSP is also involved in the heat-induced EMT process. STIP1, which is increased by IRFA, has been reported to bind to HSP to form a complex involved in the development of EMT.[25]

Immune Microenvironment

Immunotherapy is penetrating all aspects of the therapy of cancer, and the immune microenvironment (TIME) is an important factor determining the efficacy of immunotherapy.[95–97] Local treatment can alter the immune microenvironment of HCC and thus affect its efficacy.[3,98] Studies by Zerbini et al and Mizukoshi et al have shown that RFA monotherapy can activate tumor-specific T cells,[99,100] but is not sufficient to control tumor progression. Two clinical

Table 1 Autophagy and IRFA

Reference	Mechanisms	Functions	Sample	Drugs
[35]	Autophagy and expression of CD133	Autophagy increases survival and invasion	Huh-7 and SMMC7721 after IRFA	3-MA or CQ
[36]	Autophagy and expression of HIF-1α	Autophagy increases proliferation rate	HepG2 or SK-Hep-1 after IRFA; BALB/c mice after IRFA	HCQ or CQ
[37]	Expression of HSP and decreases autophagy		BALB/c mice after IRFA	HSP90 inhibitor 17-AAG
[29]	Autophagy and expression of BNIP3	Autophagy increases cell vitality, migration and invasion	HepG2 or SMMC7721 after IRFA	
[39]	HSCs, HGF and autophagy create synergies	Activated HSCs promote progression of residual HCC cells after sublethal heat treatment from autophagic survival to proliferation via HGF/c-Met signaling	MHCC97H, HCCLM3, SMMC7721, HepG2, Huh7 after IRFA	CQ and c-Met inhibitor
[41]	IRFA makes autophagy and HSP work together		Nude mice after IRFA	The combination of 17-AAG and 3-MA (autophagy inhibitor)
[38]	The ATP-AMPK-mTOR signaling pathway is involved in heat-induced autophagy	Autophagy increases proliferation and apoptosis	SMMC7721, HuH7 after IRFA; BALB/c nude mice after IRFA	CQ

Abbreviations: IRFA, insufficient radiofrequency ablation; 3-MA, 3-methyladenine; CQ, chloroquine; HSP, heat shock protein; 17-AAG, tanespimycin.

studies by Duffy et al showed that subtotal RFA combined with CTLA4 inhibitors resulted in an increase of CD8+ T cells in a tumor or peripheral blood,[101,102] and similar results have been observed in animal studies.[103] Notably, IRFA combined with PD-1 inhibitors may not yield better results. Shi et al found that complete ablation combined with PD-1 blockade therapy significantly increased CD8+ T cells, and this combination was found to achieve a better survival benefit in the experiments in vitro.[104] However, in a follow-up study, Shi et al found that IRFA triggered an increase in infiltrating myeloid cells in residual tumors and suppressed T-cell function, which ultimately blocked the effect of PD-1 inhibitors.[52] This contradicts the study by Duffy et al and may be due to differences in immunosuppressant or differences in the means of ablation. Nevertheless, the changes in TIME caused by IRFA are uncertain, and more research is needed, from clinical trials to experiments in vivo.

Hypoxia-Inducible Factor

Although hypoxia has a negative impact on tumor proliferation in some cases, however, the main aspect is to adapt the tumor cells to the oxygen and nutrient deficit, thus inducing cancer cell survival by activating autophagy, suppressing apoptosis.[105,106]

Hypoxia-inducible factor (HIF) expression is significantly elevated in the hypoxic tumor microenvironment, which contributes to mechanisms such as metabolic reprogramming, autophagy, angiogenesis, and EMT.[107–109] Tong et al simulated the survival environment of residual cells after IRFA through heating and hypoxia culture. They found that HIF-1α is dependent on TGF-β to activate downstream pathways that contribute to EMT and survival of CSCs in MHCC97H and SMMC7721.[24] Another study found that the use of heat alone also resulted in elevated HIF-1α expression,[48] confirming that IRFA can influence surviving tumor cells through both heat and hypoxia.[110,111] In addition, to adapt to the absence of oxygen and nutrient, the tumor will increase the growth of blood vessels as much

as possible. IRFA leads to more severe hypoxia,[60] and therefore, angiogenesis is more common. Three studies have shown that,[48–50] similar to other ways,[112,113] IRFA can also activate the HIF-1α-VEGF axis to enhance angiogenesis. HIF-1α has also been reported to be involved in the process of metabolic reprogramming,[114,115] while sublethal heat stress-induced O-GlcNAcylation regulates the Warburg effect in HCC cells by promoting the stability of HIF-1α,[34] suggesting that sublethal heat stress may act as a "switch" that triggers a stronger Warburg effect to drive HCC progression.

Growth Factors

Angiogenesis is associated with the growth and metastasis of tumors.[116] VEGF has been shown to play a crucial role in tumor angiogenesis. The binding of VEGF to its receptors leads to new vessel formation by inducing mitogenesis and chemotaxis of normal endothelial cells and increasing vascular permeability.[117,118] VEGF has long been shown to be highly expressed in HCC tissue.[119,120] VEGF or its receptors tends to be more abundantly expressed in liver cancer tissue after IRFA, and as a result, more tumor angiogenesis was observed.[24,46,48,49,54,57,121–123] Notably, Tan et al indicated that VEGFR-2 was not affected by temperature, whereas VEGFR-1 was significantly elevated following incomplete ablation and was strongly associated with tumor metastasis and stemness.[46]

HGF is mainly secreted by mesenchymal cells, and serum HGF levels are significantly higher in HCC patients compared to normal controls.[124,125] HGF activates downstream c-met, causing a series of downstream biological programs that ultimately inhibit apoptosis and promote cell proliferation and invasion.[126,127] Previous studies reported that the expression of HGF increased significantly in tumor tissue after heat treatment.[54] Zhang et al found that activated HSCs clustered around residual HCC cells, and HGF expression was up-regulated in the fibrous stroma, suggesting that HGF may come from activated HSCs.[42] Two studies have shown that after IRFA, HGF acts mainly through the HGF/c-met/MAPK pathway.[42,128]

TGF-β has also been found to play an important role after IRFA. In hepatocarcinogenesis, TGF- β plays a dual role as an inhibitor in the early stage, but once the cell gets rid of its cell inhibition, it will promote tumor progression in the later stage.[129–131] It was reported that TGF- β expression was significantly increased in tumor tissues of patients after RFA.[132] Similar results were reported in a study by HE et al who found that the hypoxic environment following IRFA caused increased TGF-β secretion and caused EMT via the HIF-1a/TGF-b1/Snail pathway.[24] Table 2 shows the potential relations or mechanisms of growth factors and IRFA.

Hepatic Stellate Cells and Tumor-Associated Endothelial Cells

HSCs infiltrating HCC secrete many cytokines, extracellular matrix proteins chemokines, growth factors, and consequently remodel tumor microenvironment. The level of HGF in HSC-CM was found higher than that in control medium, and Zhang et al found HGF switches autophagic survival to proliferation in residual HCC cells through the regulation of HGF/c-Met/ERK1/2 signaling from downstream autophagic axis of ATG5/Beclin1 to proliferative axis of cyclinD1.[42] In addition, the POSTN in HSC-CM promotes EMT and regulates the stemness of residual HCC cells enhancing the malignancy of HCC.[27,45]

Similar to HSCs, tumor-associated endothelial cells (TAECs) are also an important component of the microenvironment of tumor, which play a key role in angiogenesis. TAECs were activated by IRFA and the invasiveness of HCC cells was promoted when HCC cells were cultured in the conditioned medium from TAECs after IRFA.[58] Additionally, the expression of ICAM-1 in TACEs was elevated consequently activating platelets and increasing endothelial permeability which is associated with the growth and metastasis of HCC after IRFA.[10]

Therapeutic Strategies

Sorafenib is a multi-kinase inhibitor and is one of the important drugs in the systemic treatment of advanced HCC.[133,134] Sorafenib is a commonly used drug in preclinical trials involving IRFA. It has been reported to eliminate the differences in survival rates, migratory capacity, and invasive ability between heated or non-heated HCC cells, and it can inhibit heat-induced morphological changes[23,24] as well as intra-tumor angiogenesis.[30,49] Additionally, sorafenib also inhibits or

Table 2 Growth Factors and IRFA

Reference	Growth Factors and Receptors	Mechanisms	Functions
[52]	VEGF and HGF	IRFA increases expression of VEGF and HGF	Contribution to growth and metastasis of tumors
[46]	VEGFA	IRFA induced PI3K/Akt/HIF-1a/VEGFA signal transduction pathway	Contribution to the growth of tumor and increases the density of blood vessels
[47,48,55,123]	VEGF	The expression of VEGFA was increased after IRFA	Elevation of micro-vessel density
[122]	VEGF	IRFA induced CaMKII/ERK/VEGF signal transduction pathway	
[53]	EGFR	Heat stimulation induced significant activation (phosphorylation) of EGFR and had no effect on total EGFR expression	
[22]	TGF-β1	Hypoxic microenvironment triggers the pathway: HIF-1a/TGF-b1/snail	HIF-1a/TGF-b1/snail pathway enhances invasion, metastasis and anti-apoptosis
[44]	VEGFR1 and VEGFR2	VEGFR1 was found to be up-regulated along with enhanced migration after heating while VEGFR2 was down-regulated by Western blot	Promotion of the metastatic potential of HCC and stemness
[40]	HGF	HGF from HSC-CM regulated the HGF/c-Met/ERK1/2 pathway	Transition from autophagic survival of residual hepatocellular carcinoma cells after heat treatment into proliferation
[34]	EGFR	IRFA activated m^6A-YTHDF1-EGFR axis	Promoting HCC cell viability and metastasis

Abbreviations: IRFA, insufficient radiofrequency ablation; VEGF, vascular endothelial growth factor; VEGFA, vascular endothelial growth factor-A; HGF, hepatocyte growth factor; EGFR, epidermal growth factor receptor; TGF-β, transforming growth factor-β; HIF, hypoxia inducible factor.

reverses EMT,[135] however, it has been shown that IRFA can cause a significant increase in ATPase inhibitory factor 1 (IF1) expression, which can blunt the effect of sorafenib.[30]

In addition to anti-malarial effects, chloroquine has been widely reported as potential anti-cancer agent due to its blocking of autophagy.[136,137] We have said above that autophagy is a key mechanism for tumor cell survival after IRFA. Chloroquine significantly increased the apoptosis of HCC cells after IRFA and inhibited the enrichment of CSCs, and this effect was significantly enhanced by the combination of C-MET inhibitors.[37,38,40,42]

Some researchers found inflammation contributed to tumor metastasis and suppression of T-cell function.[52,138,139] Jiang et al used different doses of aspirin to treat New Zealand white rabbits after IRFA. A significant decrease was observed in the levels of serum IL-6, hs-CRP, and TNF-α, the laboratory biomarkers of inflammation, after the treatment of aspirin. Additionally, aspirin brought more survival time and the decrease of the expression of PCNA, MMP-9, and VEGF.[121]

As mentioned earlier, the effect of IRFA on the immune microenvironment is very complex. Previous studies have shown that IRFA results in enhanced systemic antitumor T-cell immune responses and tumor expression related to the increasing of dental cell infection,[140] however, the inflammation from IRFA may lead to the failure of PD-1 inhibitors.[52] A clinical trial found the immune system could potentially also recognize and kill residual cancer, and tremelimumab (anti-CTLA4 treatment) could enhance this effect,[101] which suggests that different immunosuppressants may have different effects on IRFA.

Discussion

Consistent with what has been observed in clinical practice, residual cells after IRFA are accelerating the progression of residual HCC cells through EMT, tumor stem cells, autophagy, heat shock response, immune microenvironment, and

hypoxia-inducible factors. In addition to these ways, recent studies have shown that various growth factors are also extensively involved in angiogenesis, cell survival, proliferation, and cell migration after IRFA.[46,48–51,55,123,128,132] Overall, IRFA activates residual HCC cells through multiple pathways.

The mechanisms of sorafenib,[23,24,28,30,49,141] bevacizumab,[48] melatonin,[110] chloroquine,[37,38,40,42] aspirin,[121] metformin[45,123] in suppressing residual HCC after IRFA has been successively proposed, and these drugs all work after RFA. Notably, tumor invasion and metastasis seem to occur very rapidly after IRFA, as reflected by the fact that EMT or autophagy-related molecules are rapidly elevated after RFA,[21,35,42] which prompts us to use drug treatment as soon as possible after RFA. Although we have many ways to cope with incomplete ablation after surgery, it is difficult to kill the tumor cells completely due to the combined effect of these multiple pathways. Therefore, we should emphasize the complete killing of tumor cells intraoperatively, and it has been reported that at least a sufficiently safety margin (3 mm) can reduce the chance of tumor progression,[142,143] but due to the large size, irregular shape, and "heat sink effect" of the tumor,[144–146] it is difficult to complete ablation and ensure sufficient safe margin. The evaluation of complete RFA is based on follow-up imaging or postoperative pathology, therefore, it is important to develop new techniques which can help us immediately detect residual tumors or ensure sufficient safe margin. Kan et al recently developed a "one-stop-shop" interventional oncologic technology "Intraprocedural real-time optical imaging guidance for complete tumor ablation", they can identify residual tumors in real-time during operation, to conduct repeated ablation and completely kill tumor cells.[147] In addition, the European Association for the Study recognized the value of fusion imaging (FI) which tackled the limitations of each single imaging modality (computed tomography, ultrasound and magnetic resonance imaging).[1] The FI technique provides more accurate determination and tumor location improving the rate of complete ablation.[148]

In conclusion, we summarized relevant animal and cell models for IRFA that have been used and could help subsequent researchers construct more suitable models. A series of mechanisms from EMT to growth factors were described in this review, and corresponding therapeutic strategies were summed up. Even so, IRFA is still an important reason for the progression of liver cancer. We need earlier and more effective imaging methods to evaluate early progress to facilitate repeated ablation. Considering that multiple pathways are activated, the combination of multiple targeted drugs and immunosuppressants may be an effective means. Additionally, we would like to point out that hepatocarcinogenesis is a typical multistage process and most HCC patients have underlying liver diseases which play key roles in tumor microenvironment,[149,150] therefore, diverse animal models with liver diseases (such as cirrhosis) reflecting the clinical setting more closely are needed. As far as we know, although many studies have been published in this field, there is still a lack of relevant review. It is certain that with the deepening understanding of IRFA, we will help patients after ablation achieves more survival benefits.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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