



Clinical outcomes and tumor microenvironment response to radiofrequency ablation therapy: a systematic review and meta-analysis

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Background: Radiofrequency ablation (RFA) utilizes minimally invasive high-energy current to precisely ablate tumor cells. It has been utilized in many cancer types including thyroid, lung, and liver cancer. It has been shown to provide adequate ablative margins with minimal complications; however, incomplete RFA may lead to recurrence of tumor. The underlying cellular mechanism and behavior of ablated cancer tissue is poorly understood.

Methods: A systematic review was performed, searching EMBASE, Web of Science, PubMed, and Scopus for studies published up to July 2022 and reported following PRISMA guidelines. Collection was performed by two groups of investigators to avoid risk of bias. The Cochrane Collaboration's tool was used for assessing risk of bias. We identified human, *in vivo*, and *in vitro* research studies utilizing RFA for tumor tissues. We required that the studies included at least one of the following: complications, recurrence, or survival, and took interest to studies identifying cellular signaling pathway patterns after RFA. Descriptive statistical analysis was performed in 'R' software including mean and confidence interval.

Results: The most frequent cancers studied were liver and lung cancers accounting for 57.4% (N=995) and 15.4% (N=267), followed by esophageal (N=190) and breast cancer (N=134). The most common reported complications were bleeding (19%) and post-operative pain (14%). In our literature search, four independent studies showed upregulation and activation of the VEGF pathway following RFA, four showed upregulation and activation of the AKT pathway following RFA, three studies demonstrated involvement of matrix metalloproteinases, and four showed upregulation of c-Met protein following RFA.

Conclusions: In our review and meta-analysis, we identify several proteins and pathways of interest of which are important in wound healing, angiogenesis, and cellular growth and survival. These proteins and pathways of interest may implicate areas of research towards RFA resistance and cancer recurrence.

Keywords: Radiofrequency ablation (RFA); cancer recurrence; cellular signaling pathway

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Introduction

Background

Radiofrequency ablation (RFA) is a minimally invasive intervention that utilizes a high-frequency thermal current to precisely ablate tumor cells while preserving the surrounding tissue (1). Treatment with RFA is associated with a low complication rate, low mortality, and low cost (2-4). RFA is considered an effective treatment for several cancer types. In the treatment of primary and metastatic lung tumors, RFA is shown to provide adequate ablation margins and preserved pulmonary function (5). RFA as a treatment for hepatocellular carcinoma is favored as it is known to cause minimal complications, including lack of intraabdominal adhesion, and may be considered a survival-enhancing treatment before liver transplantation (6). Researchers have extensively studied RFA of thyroid nodules and continuously proven its efficacy, safety, and cost-effectiveness with an associated long-term follow-up time and reduced tumor volume (7,8).

Rationale and knowledge gap

The exact anti-tumor mechanism of RFA is unknown. An *in vivo* study on hepatocellular carcinoma reports

increased apoptosis and heat shock protein 70 (HSP-70) expression, positively correlated with increased lymphocytic CD8⁺ T-cell invasion (9). Another study reported the upregulation of the IL-6/c-Met/HGF and VEGF pathways in insufficient RFA of tumor cells (10). Insufficient RFA remains a challenge for tumors larger than 3 cm, oftentimes resulting in recurrence and resistance to further treatment (11,12). Local recurrence rates depend on remaining ablative margins, as recurrence is found to be around the periphery of the ablation zone. Recurrent tumor cells are shown to be increasingly proliferative, neovascularized, and resistant to treatment compared to primary tumor cells (13). Studies have demonstrated the requirement of an additive pharmaceutical therapy to control tumor activity. The addition of a heat shock protein inhibitor was shown to decrease tumor size and increase overall survival *in vivo* (14). An epidermal growth factor receptor (EGFR) inhibitor was also shown to suppress malignancy in liver tumors in animal models (15).

Objective

Understanding the pathophysiological mechanism of RFA and the created tumor microenvironment may allow researchers to prevent RFA recurrence and resistance. We reviewed the literature for clinical, *in vivo*, and *in vitro* studies investigating mechanisms of RFA in various cancers such as breast adenocarcinoma, lung carcinoma, and hepatocellular carcinoma. We identified protein markers and pathway activity present following RFA treatment. We present this article in accordance with the PRISMA reporting checklist (available at <https://gs.amegroups.com/article/view/10.21037/gs-22-555/rc>).

Methods

Search strategy

The literature design of this constructed meta-analysis and systematic review was accomplished utilizing the PRISMA guidelines (16). A precise systematic screening was executed using the subsequent electronic scientific databases including EMBASE, PubMed, Web of Science, and Scopus until July 2022. The inclusion criteria of this work

Highlight box

Key findings

- We identified several proteins and pathways of interest which are important in wound healing, angiogenesis, and cellular growth and survival.

What is known and what is new?

- Radiofrequency ablation (RFA) has been proven safe and effective. However, the exact antitumor molecular mechanism is unknown.
- Several proteins and pathways of interest such as VEGF, AKT, matrix metalloproteinases (MMPs), and c-Met were identified which are implicated in wound healing, angiogenesis, and cellular growth and survival.

What is the implication, and what should change now?

- The identified proteins and pathways of interest may implicate areas of research towards RFA resistance and cancer recurrence.
- Further research may focus on the regulation of these pathways post-RFA to overcome mechanisms of resistance.

were modulated using a sequence of pertinent keywords involving [“radiofrequency ablation”, “radiofrequency thermal ablation”, “RFA”, “ablation”, “incomplete ablation”, “recurrence ablation”, “thermal ablation”, or “insufficient ablation”] and [“antitumor”, “tumor”, “cancer”, “cancerous”, “carcinoma”, “neoplasm”, “tumor destruction”, or “tumorigenesis”] and [“molecular pathway”, “signaling pathway”, “upregulation”, “downregulation”, “recurrence”, or “survival”]. A total of 362 works were identified, with 228 duplicates removed, totaling to 134 remaining works.

Inclusion and exclusion criteria

The designated inclusion criteria were formulated as follows: (I) human, *in vivo*, or *in vitro* studies that utilized RFA for tumor tissues; (II) retrospective, observational or diagnostic accuracy design; (III) reported at least one molecular or signaling pathway; (IV) stated any cancer type; (V) reported at least one of the following outcomes: tumor size change, complications, recurrence, or survival; and (VI) no restrictions for age, gender, or geographical localization. On the other hand, the exclusion criteria set was as follows: (I) narrative reviews, literature reviews, editorial materials, letter to editors, or meetings; (II) repetitive or duplicated publications; (III) insufficient or overlap data; and (IV) non-English reports.

Data extraction and manipulation

Two groups of independent researchers (LM, MHH, RE, MH) manually extracted and tabulated the clinical data from the relevant studies. These researchers worked independently to mitigate risk of bias. All studies were reviewed to ensure blinding of participants and researchers and blinding of outcomes to avoid performance and detection bias using the Cochrane Collaboration’s tool for assessing risk of bias. The extracted information was abstracted within a designed tabulated form containing the following items: the surname of the first author, the publication’s date, country/region, study design, the type of cancer, the overall sum of subjects. Moreover, the prognostic findings, survival rate, molecular pathway were reported. Any disagreements raised by the investigators are subjected to resolving by the aid of the third investigator (ET).

Statistical analysis

Wilson Score interval with continuity correction was used for confidence interval calculation. Raw untransformed proportion was reported. To pool quantitative variables, DerSimonian-Laird estimator method was carried out and raw untransformed means were reported. R package ‘metafor’ and ‘meta’ were used. Results showed significant heterogeneity with I^2 exceeding 50%, therefore, values of random effects model were selected.

Results

Characteristics of included studies

Twenty-two studies with 23 comparisons were eligible (13,14,17-36) (*Figure 1*). They were published during the period between 2006 and 2022. Of these, 68.5% (N=15) were interventional studies, while others had a retrospective study design (*Table 1*). All studies were blinded of participants and researchers and blinding of outcomes to avoid performance and detection bias. For all basic science studies, data were de-identified and clinical outcomes were blinded to researchers. The most frequent cancers studied were liver and lung cancers accounting for 57.4% (N=995) and 15.4% (N=267), followed by esophageal (N=190) and breast cancer (N=134) (*Figure 2*). The studies included 1,732 cancer patients who underwent RFA. Their mean age was 63.06 years (95% CI: 60.57–65.65), and 33% were females (95% CI: 20–49%). They followed up for an average of 33.7 months (95% CI: 22.4–44.9) (*Figure 3*). Common complications included bleeding in 19% of patients and post-operative pain in 14% of patients, as shown in *Figure 4*. RFA was administered for an average of 274 seconds (n=14). Insufficient data were available to measure percentage of tumor shrinkage or quantitative recurrence data after RFA. Biopsy of tumor occurred during procedure and patient follow up time was 33 months (n=9). Tumor activity was examined 2 minutes to 72 hours after RFA; changes in molecular environment over time were poorly reported.

In vitro studies

Nine studies investigated cellular mechanism of RFA on cancer cells (14,18,20,24,36-40). They included 4 types

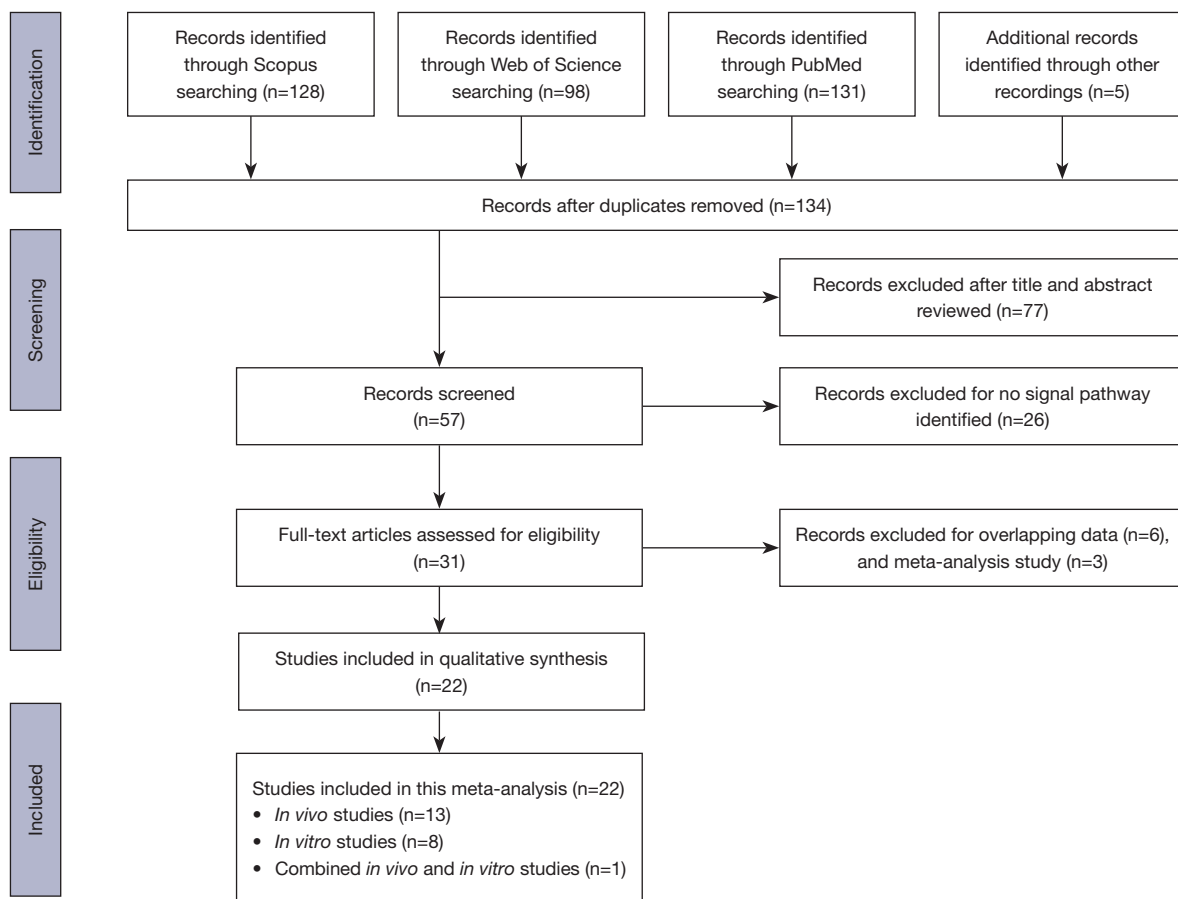


Figure 1 Workflow for search process.

of cancer: breast, liver, lung, and bladder using the cell lines demonstrated in *Table 2*. Cellular mechanisms of upregulated and downregulated pathways and proteins *in vitro* are shown in *Figure 5*.

In vivo studies

Fourteen studies used animal models to investigate the cellular effects of RFA using cell lines from eight liver, three lung, two breast, and one bladder (13,14,18-21,24,25,28,33,34,39-41) (*Table 3*). Follow-up period was not reported. Cellular mechanisms of upregulated and downregulated pathways and proteins *in vivo* are shown in *Figure 6*. Synergistic effect of RFA when combined with some drugs is demonstrated in *Table 4*.

Literature review of deregulated pathways and markers

Understanding the mechanism of RFA and the created

tumor microenvironment may allow researchers to prevent RFA recurrence and resistance. It may help surgeons understand potential complications should the site of the RFA need to undergo further operative treatment, such as increased bleeding due to increased peripheral vascularity as a wound healing mechanism. In this review, we will detail the upregulated pathways in the tumor microenvironment after RFA procedure.

VEGF signaling pathway

As cancerous cells are known to induce angiogenesis, one well-studied pathway in cancer biology is that of the VEGF pathway. In our search, we found four independent studies which found the upregulation and activation of the VEGF pathway following RFA. Most recently, a 2020 US-based study looking at mice with induced hepatocellular cancer treated by RFA were found to have significantly elevated mRNA levels of VEGFA and HIF-1 α by quantitative polymerase chain reaction (qPCR) compared to that of non-

Table 1 Characteristics of enrolled clinical studies in human

Author, year (reference)	Country/region	Study design	Cancer type	Sample size	Mean age, years	Female, %	Mean follow-up, months
Kong, 2022 (17)	China	Retro	GI (pancreas, GB, liver)	150	62.2	25.3	10.7
Wang, 2021 (18)	China	Int	Liver	120	61.0	23.3	60.6
Li, 2021 (19)	China	Int	Lung	42	58.5	81.0	NA
Zhou, 2021 (20)	China	Int	Lung	25	NA	NA	NA
Zhang, 2020 (13)	China	Int	Liver	9	NA	NA	NA
Liao, 2020 (21)	Israel	Int	Liver	216	NA	NA	NA
Wang, 2020 (22)	China	Int	Liver	8	NA	NA	NA
Tan, 2020 (36)	UK	Retro	Esophagus	145	66.6	13.8	18.0
Januszewicz, 2020 (23)	UK	Retro	Esophagus	45	67.0	15.6	49.2
Zhou, 2019 (24)	China	Int	Bladder	5	NA	NA	NA
Kumar, 2018 (25)	USA	Int	Liver	145	NA	NA	NA
Abbas, 2017 (26)	UK	Retro	Breast	11	53.0	100.0	43.0
Hyun, 2016 (27)	South Korea	Retro	Liver	14	63.1	14.3	45.3
Yang, 2016 (14)	China	Int	Breast	80	NA	NA	NA
Moussa, 2014 (28)	USA	Int	Liver	68	NA	NA	NA
Nour-Eldin, 2011 (29)	Germany	Retro	Lung	200	59.7	54.0	NA
Chang, 2011 (30)	Taiwan	Int	Liver	79	65.8	21.5	NA
Otto, 2010 (32)	Germany	Int	Liver metastasis (CRC)	28	64.0	28.6	26.7
Dal Bello, 2010 (31)	Italy	Retro	Liver	207	70.0	37.2	36.0
Solazzo, 2010 (33)	USA	Int	Breast	43	NA	NA	NA
Nijkamp, 2009 (34)	The Netherlands	Int	Liver metastasis (CRC)	8	NA	NA	NA
Cho, 2006 (35)	South Korea	Int	Liver	84	NA	NA	16.0

Retro, retrospective study; GI, gastrointestinal tumors; GB, gall bladder cancer; Int, intervention study; NA, unknown; CRC, colorectal cancer.

treated mice. Accordingly, both VEGFR2 and VEGFR1 transcription levels were significantly elevated, with the latter increasing 4-fold. Using sunitinib, an FDA-approved multi-targeted receptor tyrosine kinase (RTK) anti-cancer medication, the authors showed that the therapy was able to downregulate VEGF signaling, decrease VEGFR2 and CD31 (a common marker of vascular differentiation) expression, as well as prolong mice life span and decrease its tumor size post-RFA (41). Another 2020 study analyzing tissue samples of patients with liver cancer who underwent insufficient RFA followed by hepatectomy or solely hepatectomy found increased protein expression of VEGFA in the post-RFA group. Similarly, this study also

found a 2-fold increase in CD31 protein expression (13). A distinct work in both rats and mice demonstrated a 2-fold increase in VEGF expression in mice following RFA when compared to mice undergoing a sham procedure (for vehicle control). Interestingly, they found that a STAT3 inhibitor was able to significantly reduce circulating VEGF levels in the RFA-treated mice, yet not to the extent of the sham-treated mice, suggesting that RFA, at least in part, increases VEGF via a STAT3 signaling pathway (25). One final work investigating the VEGF pathway following RFA in two separate animal models (rat and mice) with colorectal liver metastases also found the pathway to be upregulated (34). Interestingly, Nijkamp *et al.* also analyzed the expression

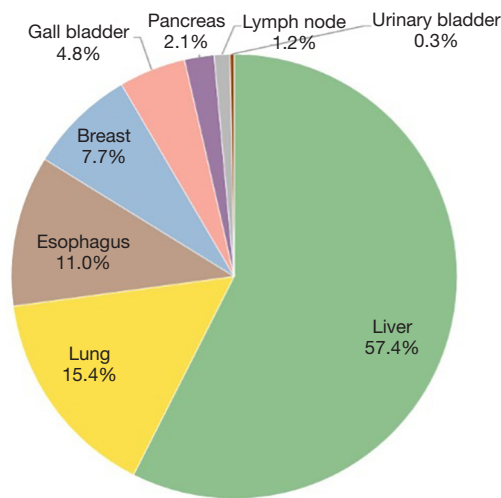


Figure 2 Frequency of patients underwent RFA therapy across various types of cancer. RFA, radiofrequency ablation.

of pro-angiogenic genes in the same animal by analyzing a reference (control) zone and a tissue-ablated zone, and unsurprisingly found no HIF-1 α and HIF-2 α staining in the reference zone but strong staining for HIF-1 α and HIF-2 α staining in the ablated zone (34). The Netherlands-based study also found that the tumor-bearing mice five days post-RFA displayed neovascularization with vessels four to five times the size of normal sinusoidal diameter (34). Finally, they found that the usage of a VEGF receptor inhibitor significantly limited post-RFA tumor growth when compared to post-RFA untreated animals (34). Unsurprisingly, the four works analyzing VEGF with respect to RFA for the treatment of cancer all indicate upregulation of the pro-angiogenic pathway. Several anti-VEGF pathways medications have been approved, many of which have been recommended for the treatment of cancers, including renal cell carcinomas and gastrointestinal stromal cancers. Sunitinib, which was able to effectively reduce post-RFA tumor size and prolong mouse life span, as well as other FDA approved medications which possess established safety profiles warrant further study.

AKT signaling pathway

Another studied molecular pathway following RFA is that of the AKT signaling pathway, which is responsible for a multitude of trophic processes including cell proliferation, survival, growth, and metabolism. AKT-pathway mutations have been reported in a multitude of cancers and are specifically associated with less differentiated thyroid

carcinomas (42). In 2016, it was found that lung cancer cells following insufficient RFA regrew via PI3K/Akt/HIF-1 α signals (43). Since then, three new works (for a total of four studies) have each independently shown the AKT pathway to be upregulated following RFA. Most recently, a 2021 work using a mouse model with hepatocellular carcinomas found three major proteins in the AKT pathway, including p-PI3K, p-AKT, and p-mTOR, to each be significantly upregulated when cells had undergone insufficient RFA. Interestingly, their work demonstrated that the use of all-trans retinoic acid, an FDA approved and well-accepted anti-cancer medication, could normalize the levels of p-PI3K, p-AKT, and p-mTOR to that of the control (primary tumors) as well as suppress tumor regrowth, suggesting a link between the two (18). A separate work in mice with metastatic lung cancer subject to RFA found that residual tumor expressed increased protein levels of AKT, HIF-1 α , and CD34 when compared to that of the reference (control) zone which was not ablated. The authors also demonstrated that the use of osimertinib, a signal-targeted therapy against RTKs, could effectively minimize the upregulation of the aforementioned proteins (AKT, HIF-1 α , and CD34) as well as significantly decrease tumor growth (40). The final two studies also analyzed the effect of RFA in hepatocellular carcinomas (36,39). One study indirectly found RFA to upregulate the AKT pathway. In both patient tissue samples as well as cultured cell lines, the study demonstrated that miR-103 levels were upregulated in hepatocellular carcinomas of patients undergoing thermal ablation. Then, to demonstrate the link between RFA and the AKT signaling pathway, cells which were treated with miR-103 were found to have upregulated protein expression levels of p-AKT and its downstream AKT-mediators (CyclinD1, p21, Bim, Fasl). Interestingly, the work bolstered the importance of the AKT pathway by analyzing The Cancer Genome Atlas and determined miR-103 upregulation in hepatocellular cancers in comparison to normal patient tissues (36). Finally, one work found that cultured cancer cells treated with insufficient RFA had increased p-AKT and p-ERK1/2 expression compared to non-treated cells. Attractively, they found that though heat-treated cells demonstrated increased proliferation (6.4%), migration (33.2%), and invasion (44.1%) compared to their non-treated counterparts, the effect was completely abated when treated with a specific p-ATK inhibitor, LY294002 (39). Since the AKT pathway mediates a multitude of cancers (including that of the thyroid) and the above-mentioned studies consistently demonstrate

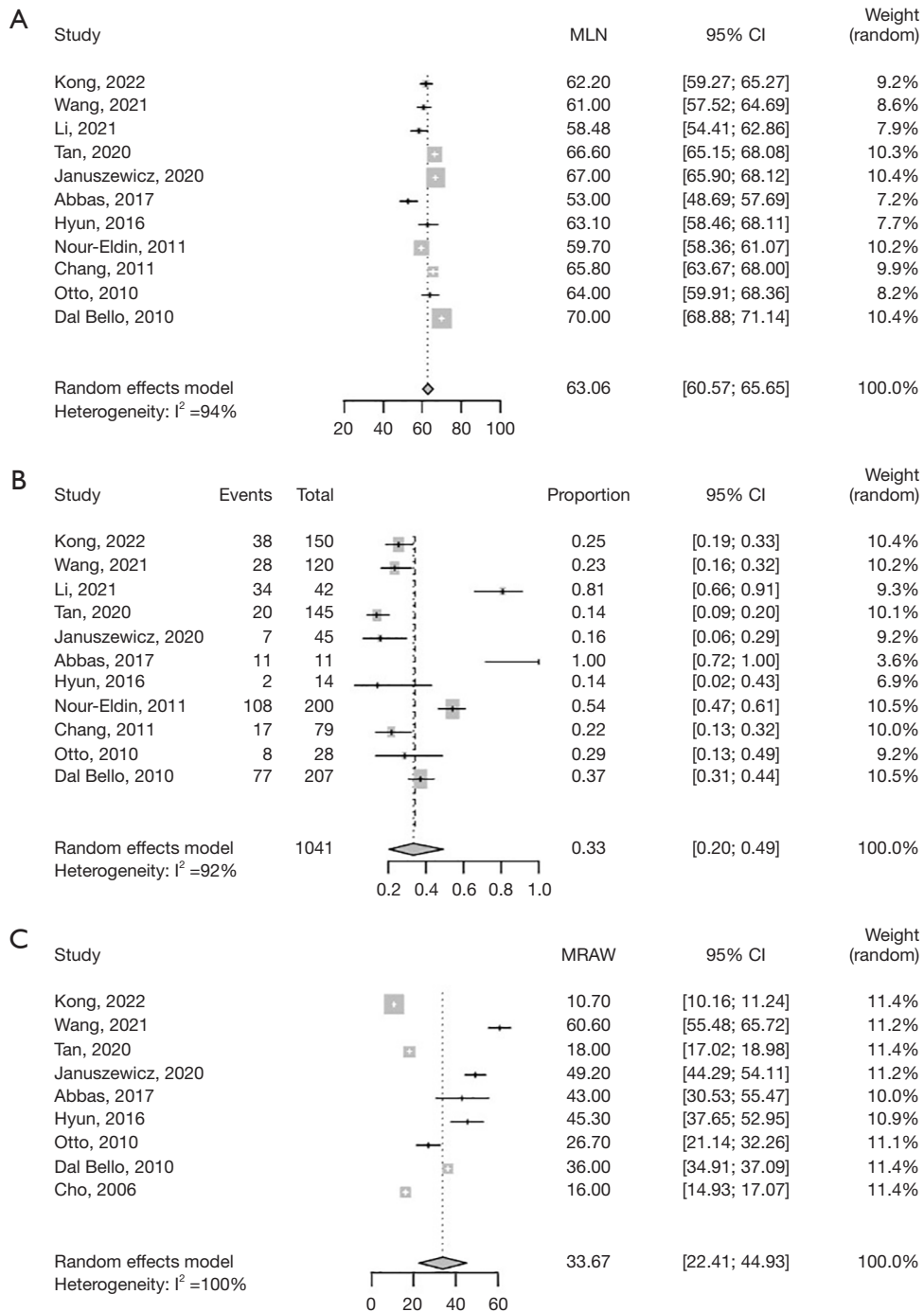


Figure 3 Pooled demographic characteristics of the study population. One arm meta-analysis was conducted. (A) Age of patients per study. (B) Pooled proportion of female patients per study. (C) Follow-up period following RFA procedure. CI, confidence interval; RFA, radiofrequency ablation.

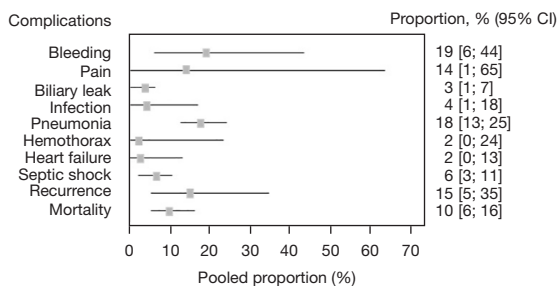


Figure 4 Pooled frequency of complications following RFA therapy in cancer patients. CI, confidence interval; RFA, radiofrequency ablation.

the upregulation of the AKT pathway following RFA in hepatocellular carcinomas, medications exploiting the AKT pathway may be of potential interest for patients undergoing RFA of the thyroid.

MMPs

Few studies have analyzed common biomarker proteins following RFA, including the MMP class. The MMPs are a class of endopeptidases which, analogous to a double-edged sword, mediate both the normal and pathological degradation of extracellular matrix proteins. In specific,

Table 2 Cancer and cell lines used for RFA procedure

Author, year (reference)	Country	Cancer	Cell lines	Drug enhancement
Wang, 2021 (18)	China	Liver	HepG2, Huh7	ATRA
Zhou, 2021 (20)	China	Lung	NCI-H520, NCI-H226, A549	Anlotinib
Wang, 2021 (40)	China	Lung	nci-h1975	Osimertinib
Jia, 2020 (37)	China	Liver	hepG2	
Tan, 2020 (36)	China	Liver	hepG2	
Zhou, 2019 (24)	China	Bladder	T24, 5637, RT4	SB431542 - STAT inhibitor
Tong, 2017 (38)	China	Liver	MHCC97H, SMMC7721	
Yang, 2016 (14)	China	Liver, breast	MCF-7, HTB-22, ATCC; HEP-1, HTB-52, ATCC	Quercetin – HSP inhibitor
Dong, 2013 (39)	China	Liver	SMMC7721, Huh7	PI3K/Akt inhibitor LY294002, or ERK1/2 inhibitor PD98059

RFA, radiofrequency ablation; ATRA, all-trans retinoic acid; STAT, signal transducer and activation of transcription; HSP, heat shock protein.

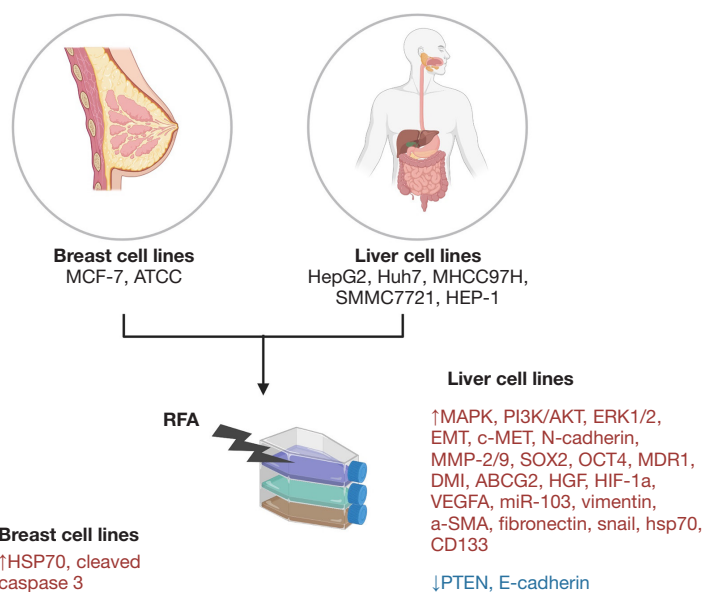


Figure 5 Cellular pathway activity after RFA *in vivo*. Activated pathways in red and inhibited pathways in blue. RFA, radiofrequency ablation.

Table 3 Animal model studies for cancer used RFA procedure

Author, year (reference)	Country	Cancer	Time (s)	Temp (°C)	Type of animal	No of groups	Total sample size	Injected cell lines
Wang, 2021 (18)	China	Liver	300	65	Mice	2	7	HepG2
Li, 2021 (19)	China	Lung	–	–	Mice	4	42	C57BL/6
Zhou, 2021 (20)	China	Lung	120	70	Mice	4	65	h520, h226
Wang, 2021 (40)	China	Lung	600	47	Mice	2	20	BalB/c
Qi, 2020 (41)	USA	Liver	60	80	Mice	4	55	MTD2
Zhang, 2020 (13)	China	Liver	600	50	Mice	3	NA	HepG2, Huh7
Liao, 2020 (21)	Israel	Liver	300	70	Mice	NA	216	BalB/c, C57BL6
Zhou, 2019 (24)	China	Bladder	300	70	Mice	2	–	BalB/c
Kumar, 2018 (25)	USA	Liver	300	70	Mice, rats	8	142	C57BL6, F344
Yang, 2016 (14)	China	Breast	300	70	Rats	8	80	R3230
Moussa, 2014 (28)	USA	Liver	300	70	Rats	NA	68	R3230
Dong, 2013 (39)	China	Liver	300	47	Mice	NA	NA	BalB/c
Solazzo, 2010 (33)	USA	Breast	300	70	Rats	5	110	R3230
Nijkamp, 2009 (34)	The Netherlands	Liver metastasis (CRC)	50	–	Mice, rats	2	16	C26, CC531

RFA, radiofrequency ablation; Temp, temperature; CRC, colorectal cancer; NA, not available.

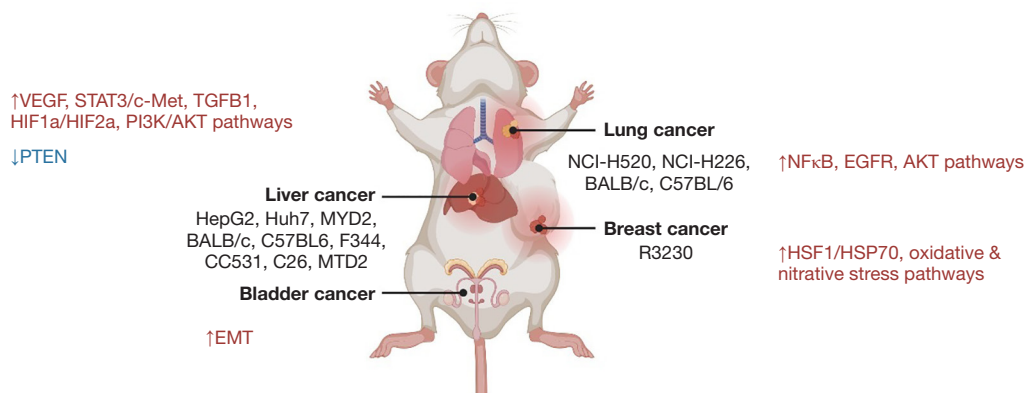


Figure 6 Cellular pathway activity after RFA *in vivo*. Activated pathways in red and inhibited pathways in blue. RFA, radiofrequency ablation.

MMP-2 (digests collagen) and MMP-9 (digests collagen and serves an essential role in leukocyte migration) have been associated with a plethora of cancers and are found circulating at elevated levels in patients with thyroid cancers (24,44). We found three studies investigating MMP-2 and MMP-9, all of which demonstrated elevated transcription or protein levels following RFA in cultured hepatocellular carcinoma cells (14,36,39).

One work demonstrated that radiofrequency ablated-patient tissue samples displayed elevated miR-103 levels, and subsequently stimulated recurrent hepatocellular carcinoma cells *in vitro* with miR-103. They found stimulated-cultured cells had significantly elevated MMP-2 promoter activity as well as elevated MMP-9 levels (36). A separate study directly comparing untreated cells and those treated by RFA also corroborated this notion,

Table 4 Role of drug enhancement on RFA outcomes

Author, year (reference)	Cancer type	Animal model	Injected cell lines	Drug enhancement	Regulatory effect
Wang, 2021 (18)	Liver	Mice	HepG2	ATRA	ATRA triggers apoptosis and suppresses TICs (CD133 ⁺ and EpCAM ⁺) by inhibiting PI3K/AKT pathway
Li, 2021 (19)	Lung	Mice	C57BL/6	Melatonin	Analysis of residual tumor after combined treatment revealed depressed activity of MAPK, NF-kappa B, Wnt, and Hedgehog pathways and upregulated P53 pathway in tumors, which was in line with the inhibited tumor growth
Zhou, 2021 (20)	Lung	Mice	h520, h226	Anlotinib	Treatment with anlotinib + RFA inhibited receptor tyrosine kinase or the MPAK/PI3K-AKT pathway kinases
Wang, 2021 (40)	Lung	Mice	BalB/c	Osimertinib	OSI inhibited the EGFR/PI3K/AKT pathway and induced apoptosis in thermotolerant NCI-H1975/OSIR cells
Qi, 2020 (41)	Liver	Mice	MTD2	Sunitinib	Sunitinib represses RFA induced PD-1 and PD-L1 upregulation
Zhang, 2020 (13)	Liver	Mice	HepG2, Huh7	Amarogentin	The expression levels of CD133, VEGFA, Dll4, and Notch1 in induced RFA tumor tissues were decreased by amarogentin, and phosphorylated p53 levels were increased
Kumar, 2018 (25)	Liver	Mice, rats	C57BL6, F344	Inhibition of STAT3 with either S3I-201 or micelle-encapsulated curcumin	STAT3 overexpression after RFA led to increase in SAA1, APCS, TIMP1, SERPINAS, CYCLIN D1, P21, SOCS3
Moussa, 2014 (28)	Liver	Rats	R3230	Bortezomib - HIF-1 α inhibitor	Upregulation of HIF-1 α after RFA ablation
Dong, 2013 (39)	Liver	Mice	BalB/c	PI3K/Akt inhibitor LY294002, or ERK1/2 inhibitor PD98059	Insufficient RFA may promote the EMT of HCC cells through overexpression of Akt and ERK signaling pathways

RFA, radiofrequency ablation; EMT, endothelial-mesenchymal transition; HCC, hepatocellular carcinoma.

finding elevated MMP-2 and MMP-9 protein levels as well (39). The final work examined the effect of the hypoxia-induced environment that RFA inevitably prompts, analyzing hepatocellular cells grown in hypoxic versus normoxic conditions following insufficient RFA. Interestingly, both MMP-2 and MMP-9 protein expression levels were elevated in the post-ablation hypoxic conditions group when compared to the normoxic conditions, strongly suggesting hypoxia-induced MMP-2 and MMP-9 expression (14). By the very nature of their function, MMPs are not merely associated with thyroid cancer overall but also associated with its increased metastasis (45). Considering the above, elucidating the

association between MMP-2 and MMP-9 in RFA-ablated thyroid tissue may minimize the risk of thermal-induced malignancy and metastasis.

c-Met (tyrosine-protein kinase Met)

C-Met, also referred to as tyrosine-protein kinase Met, is a protein encoded by the *MET* gene which is known to play a role in invasive growth, mitogenesis, and morphogenesis (46,47). In specific, c-Met is known to play a role in the development of cancer by activating oncogenic pathways (e.g., PI3K, STAT3) and promote angiogenesis (48). With respect to RFA, we found that c-Met has been moderately studied with a total of four works. The first work utilized

human hepatocellular carcinoma cell lines with insufficient heat/RFA and analyzed c-Met at both the transcription and translation levels. Interestingly, they found similar levels of c-Met following RFA sequencing but a significant difference in c-Met expression [using quantitative reverse transcription polymerase chain reaction (RT-qPCR)] between the heat/RFA treated cells and non-treated cells. Similarly, they found that treated and untreated cells had similar c-Met protein levels by immunohistochemistry, but significantly more c-Met expression in the heat/RFA-treated cells upon western blotting. Within the same experiment, they found heat/RFA-treated cells to exhibit higher rates of proliferation, colony formation, and migration than non-treated cells. To ascribe this finding to the c-Met pathway, the authors treated heat/RFA-cells with a c-Met specific inhibitor (SU11274) which was able to effectively neutralize the post-RFA proliferative and colony forming (but not the migratory) effect in both a time-dependent and dose-dependent manner. Finally, with elevated levels of the c-Met specific inhibitor, heat/RFA-treated cells displayed lower proliferation rates than the control (37). Another 2020 study using an intrahepatic metastatic mouse model found that RFA induced metastatic tumor number, cellular proliferation, and intra-tumoral neovascularization when compared to a control sham procedure (needle placement without heating). Treatment with PHA-665752, a specific c-Met inhibitor, reduced aforementioned intrahepatic metastatic parameters (tumor number, cellular proliferation, and intra-tumoral neovascularization) following RFA to that of the control sham procedure group (21). The third work utilized a mouse model with induced hepatocellular cancer treated by RFA to show elevated (~2.5× from control) c-Met mRNA expression in RFA-treated mice which was effectively neutralized in mice treated with both RFA and an FDA-approved multi-targeted RTK drug (sunitinib). RFA with sunitinib treatment, the authors found, could synergistically significantly decrease tumor size and prolong mouse life span (41). Finally, a separate study by Zhou *et al.* using anlotinib, a RTK inhibitor, combined with RFA could minimize tumor volume more effectively than RFA alone in two separate mouse lung cancer models (lung adenocarcinoma model and lung squamous cell carcinoma model) (20).

Other players

Beyond the major pathways and proteins described above,

several genes, proteins, and pathways analyzed with respect to the microenvironment following RFA have yet to be corroborated by further investigation. The following describes the remaining ‘players’. A 2021 work found that mice injected subcutaneously with Lewis lung cancer cells and treated with RFA had elevated Foxo3, ERK2 (2 mediators within the MAPK signaling pathway), and p53 (mediator within the p53 signaling pathway) protein levels. Compared to a sham control group, the authors conversely found decreased protein levels of C-myc and Beta-catenin, two mediators of the Wnt signaling pathway (19). A separate work investigating the use of RFA as a management modality for bladder cancer in lieu of serious radical cystectomy complication found that patient-derived bladder cancer, which had been previously injected into a mouse, tumor volume could be significantly reduced with RFA when compared to a solvent control. Furthermore, they found that synergistic treatment of RFA and SB431542, a specific TGFβ/Smad pathway inhibitor, could significantly reduce tumor volume more than RFA alone, suggesting a growth-promoting signaling pathway inhibition a potential combinatory mechanism against post-RFA tumor re-growth (24). Two works investigated the use of RFA in breast cancer (14,33). Yang *et al.* showed that RFA along with a heat shock protein inhibitor could slow breast adenocarcinoma tumor growth as well as increase overall survival (26.5±3.4 d) compared to RFA alone (17.6±2.5 d) in a rat model. Their study concluded that HSP70 plays a significant role in the growth of solid breast tumors following RFA (14). The other work compared RFA alone versus intravenous liposomal doxorubicin (an anti-cancer medication) with RFA (combined therapy) on apoptosis-induction in mammary adenocarcinomas. The US-based work found that combination therapy increased cleaved caspase-3 levels (a proxy for apoptosis) more than RFA alone, potentiating chemotherapies, specifically cellular oxidative and nitrate stress inducing therapies, a plausible adjuvant alongside RFA (33). Interestingly, a different work also investigated the synergy of RFA with liposomal doxorubicin (28). Their work found that rats with subcutaneous R3230 tumors treated with RFA increased HIF-1α and HSP70 expression in time and temperature-dependent manners. Attractively, they found that RFA with liposomal doxorubicin significantly reduced HIF-1α rim thickness (125.8±28.2 *vs.* 48.4±11.8 μm) and total HIF-1α index (48.5±15.9 *vs.* 6.5±1.4) when compared to RFA alone (28).

Discussion

Key findings

To our knowledge, this study is the most comprehensive analysis of patient outcomes and tumor tissue after a RFA procedure. Our study demonstrated that RFA of tumor tissue is safe and with minimal complications across all tumor types. While complications vary for each type of cancer, such as risk of hoarseness accompanying the risk of RFA of thyroid nodules, bleeding and post-operative pain remain the most common complications, across each cancer type, consistent with our study (49).

Strengths and limitations

Of note, our study had several limitations. Tumor size pre- and post-RFA procedure was not measured. Our study included many types of cancer, all of which exhibit different tumor behavior and therefore potential complications, decreasing the generalizability of our study. Additionally, several data values were unable to be collected, as the research on this given topic is limited. Despite its limitations, our study highlights an area of study that may benefit from robust research activity.

Comparison with other studies

RFA uses thermal energy to destroy tumor tissue in its direct path; however, the intensity of thermal energy decreases at the periphery of the electrode target area. Concern has been raised for a potentially complicated wound healing mechanism in the periphery of the RFA target. Insufficient RFA has been shown to demonstrate recurrence of neoplasms and alteration of normal cellular behavior (50). Suboptimal heat exposure promotes angiogenesis through VEGF pathway by promoting hypoxia-inducible factor-1 α and VEGF- α (51). Insufficient RFA has been also shown to upregulate the AKT pathway, promoting neoplastic proliferation and survival through the epithelial-mesenchymal transition pathway, driving cancer metastasis (39). Additional important players found in insufficient RFA of cancer tissue include c-Met, a tyrosine kinase receptor that upregulates embryogenesis, potentially implicating a mechanism that may promote novel neoplastic growth after the RFA procedure, and MMPs, which facilitate wound healing and tissue remodeling following injury, which establish a peripherally reactive cellular environment after RFA (37,52).

Explanation of findings

Decreasing perfusion to target tissue may be a mechanism to decrease the risk of bleeding and hemorrhage such as clipping of vessels, balloon occlusion, or embolization. Risk of injury to adjacent structures is also a concern; studies have monitored temperatures near important structures along the periphery of the ablated tissue to avoid damage (53).

Implications and actions needed

In our review, we outline several pathways and proteins of interest that demonstrate increased angiogenesis, cellular growth, and neoplastic survival. Inhibitors of the aforementioned pathways may be investigated and utilized in further treatment to prevent tumor recurrence after RFA.

Conclusions

The exact anti-tumor mechanism of RFA is unknown, especially in the periphery of the ablated target. Understanding the tumor microenvironment after tumor RFA may help researchers identify mechanisms of resistance. In our review and meta-analysis, we identify several proteins and pathways of interest such as VEGF, AKT, MMPs, and c-Met, of which are important in wound healing, angiogenesis, and cellular growth and survival. Further studies may explore pharmacologic and biologic interventions that may inhibit the pathways to decrease rates of tumor recurrence and growth after RFA.

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