Chapter 7

Minimally Invasive Therapies for Hepatocellular Carcinoma: Mechanisms of Local Control and Systemic Immunologic Response

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72275

Abstract

Minimally invasive treatments for hepatocellular carcinoma (HCC) are a cornerstone in the management of this challenging disease. For many years, percutaneously guided ablative techniques, such as radiofrequency ablation (RFA), cryoablation, and microwave ablation (MWA), have successfully treated many different solid malignancies including HCC. Since the initial implementation of these ablative techniques, there have been many advances in the design, technique, and patient selection as well as investigation into the body's response to treatment. The mechanisms of thermal-based ablative techniques, advantages and disadvantages of each technique, subsequent immunologic response following ablation, and advances in care that utilize combination therapy to potentiate the immunologic response creating a robust and long-term immunity to HCC are outlined in this chapter.

Keywords: hepatocellular carcinoma (HCC), immunotherapy, immunologic, response, immune, cancer, carcinoma, oncology, radiofrequency, microwave, ablation, cryoablation

1. Introduction

Hepatocellular carcinoma (HCC) is the most rapidly increasing type of cancer in the United States due to viral hepatitis and various forms of liver cirrhosis. HCC is resistant to traditional chemotherapy and often is not amenable to surgical resection due to factors involving the primary tumor or patient comorbidities [1, 2]. Thus, minimally invasive therapies for the treatment of malignant liver tumors have become a cornerstone of treatment. These minimally invasive techniques, including radiofrequency ablation (RFA), microwave ablation (MWA),

and cryoablation (cryo), have been shown to have distinct advantages over traditional treatment methods. These methods are not only able to locally control the malignancy through cellular necrosis and apoptosis but also potentially trigger systemic immune responses [3–6]. Additionally, these minimally invasive techniques offer other advantages such as lower morbidity, preservation of healthy tissues, lower cost, and decreased hospitalization time relative to surgical resection [5]. In this chapter, the mechanisms, advantages, disadvantages, synergism, and immunologic responses to the techniques outlined above are discussed.

2. Radiofrequency ablation

2.1. Overview

Radiofrequency ablation (RFA) is a minimally invasive technique used to thermally ablate targeted lesions in a variety of tissues. RFA is performed by percutaneously inserting one or more probes using various forms of image guidance, such as computed tomography (CT) or ultrasound (US). RFA may also be performed through other approaches such as laparoscopy and open surgery. The number of probes used is based on multiple factors such as the size of the lesion, the impedance of the targeted region, and surrounding structures such as blood vessels and lymphatic channels. In addition to the placement of RFA probes, one or more grounding pads are also placed on the patient. These grounding pads are located at a distant site from the probes. For example, a common practice is to place multiple grounding pads on both thighs when using RFA to ablate lesions located in the liver. Once the placement of the RFA probes has been confirmed with image guidance, an alternating current is generated by a power source between the probes and the grounding pads. This alternating current creates the thermal ablative region by causing ions to oscillate, generating frictional heat. RFA can reliably generate temperatures of 60–100°C in the targeted region leading to focal hyperthermic injury to the nearby cells. When temperatures reach 60°C or higher, instant cell death occurs, and at temperatures above 100°C, charring of surrounding tissues occurs. These two temperature points are crucial to the procedure because the operator can be certain that cell death has occurred in the regions >60°C, but it is also important to monitor the temperatures so that they do not increase too quickly or reach >100°C. If the temperature becomes too high, charring of the tissues occurs, which increases the impedance significantly and causes the technique to lose efficacy by diminishing the ablative zone substantially [7]. Additionally, at temperatures above 110°C, vaporization of the tissues occurs. Vaporization also increases the impedance of the tissues limiting the ablative zone [8].

The ablated area with RFA can be divided into three zones: central, transitional, and the unaffected surrounding parenchyma [8]. The central zone is the area directly surrounding the RFA probe. In this zone, the temperatures are the highest, typically >50–60°C, leading to coagulative necrosis of the cells in this region. The cells in this zone immediately undergo irreversible injury through protein denaturation of both the cytosolic, nucleic, and mitochondrial enzymes leading to coagulative necrosis. In addition to protein denaturation, the cell membrane integrity is also compromised. The higher temperatures in the central zone lead

to changes in fluid permeability through destruction of the membrane actin filaments. These membrane changes result in an intracellular fluid shift and subsequent cytolysis [5, 8].

Cells in the transitional zone are heated through conductive heat transfer from tissues in the central zone. This conductive heat transfer produces a sharp temperature gradient with average lower temperatures ranging from 40 to 45°C [5]. Cells within the transitional zone experience thermal injury, but since temperatures of 50°C are not reached, these cells do not undergo immediate cellular death [9]. Rather, the cells' metabolic processes and DNA repair mechanisms are impaired, which trigger specific changes that eventually lead to apoptosis or eventual cellular recovery. Other proposed mechanisms of cellular death include ischemia from vascular damage, reperfusion injury, and cytokine release and subsequent immunologic response to the damaged cells. Due to these changes, a complete response to ablation in this region will take several days to fully develop. This region also undergoes reactive hyperemia in response to the damage. The combination of hyperemia and increased cellular susceptibility creates a favorable environment to use liposomal chemotherapeutics. Liposomal chemotherapeutics will accumulate in the region due to the hyperemia and have increased activity on the already susceptible tumor cells. Since very few of the cells in this region are completely denatured, the transitional zone plays a critical role in the immunologic response, which will be discussed in more detail [5, 6, 8].

Surrounding parenchyma is not left totally unaffected by RFA. While the cells within this zone will not undergo cellular changes, necrosis, or apoptosis, there are several processes that will occur. There is an upregulation of various factors, presentation of antigens to antigen-presenting cells (APCs), and stimulation of the immune system, which will be discussed more in depth in later sections. Additionally, hyperemia occurs which can result in reperfusion injury [5, 6].

All of the above processes are dependent on a multitude of different factors such as the tumor composition, the surrounding parenchyma, the rate at which the energy is applied, and surrounding anatomic structures. The majority of the data on the effects of hyperthermia have been generated from literature on low-temperature hyperthermia that was applied uniformly over longer periods.

2.2. Patient selection

Traditionally, hepatic resection (HR) has been regarded as the first-line treatment for HCC, and RFA was typically reserved for patients with non-resectable disease. However, RFA has become a first-line treatment for early-stage HCC in patients with non-resectable disease, metastatic disease, recurrent HCC after HR, and for patients who are unable or are unwilling to undergo surgery [10]. RFA is best used in patients who have a solitary nodule <5 cm measured in the greatest dimension or less than three nodules all measuring <3 cm in the greatest dimension. RFA is most effective when treating HCC lesions that are ≤2 cm measured from the largest dimension. The reason it is more effective in these smaller lesions is that ablation margins of >4–5 mm can be easily obtained [1, 10]. Histologic and prospective studies have shown that the sensitivity of CT for detecting remnant neoplasm is anywhere between 36 and

44% [11, 12]. Thus, the clinician cannot readily rely on imaging to confirm that the lesion has been fully treated during or after the procedure making pre-procedure planning and patient selection crucial. Meta-analysis and systematic reviews have also shown that the efficacy of RFA and HR when used to treat lesions <5 cm is similar. There is no difference in 1-year overall survival; however, there is a difference in the 3- and 5-year survival. HR offers greater 3- and 5-year survival when compared to RFA as well as 1-, 3-, and 5-year disease-free rates. It has been postulated that these findings are due to how each treatment method works. With RFA, the primary lesion is directly targeted with minimal damage to the surrounding tissues, which may leave satellite lesions that would have been removed with HR. Additionally other factors come into play with RFA such as the shape and distribution of the ablation zone. However, RFA has been shown to have fewer complications during and after the procedure, shorter hospital stays, and is considered safer and less invasive than HR. While HR has a significant role in the treatment of HCC, there are limited studies comparing RFA to HR [10, 13, 14]. At the current time, it cannot be confirmed which treatment is superior to the other in treating early-stage HCC. It is up to the treating clinician to determine which treatment is best. RFA should be considered as a first-line treatment in specific patients with small solitary lesions <5 cm; patients with less than three lesions that are <3 cm; patients with non-resectable, metastatic, or recurrent disease; patients who elect for non-operative or a minimally invasive approach; and patients who are non-operative due to medical comorbidities [10].

2.3. Advantages and disadvantages of RFA

Of all the thermal ablative techniques for treating HCC, RFA has been the most researched. There are many technical advantages using RFA for the treatment of HCC. The most obvious advantage of RFA as well as other thermal ablative techniques is the ability to treat a wide variety of patients while sparing normal liver parenchyma.

However, an important consideration with RFA and other thermal ablative techniques is the "heat sink effect" of surrounding anatomic structures. The heat sink effect is caused when the desired ablative region contains or is abutted by larger vessels that result in heat dissipation. This dissipation can ultimately lead to temperatures not reaching cytotoxic levels in the lesion [15–17]. Animal studies have shown that the heat sink effect is not significant until the vessel diameter is ≥3 mm [15]. Additional studies have shown a clinically significant increase in tumor recurrence when abutted by a vessel at least 3 mm in diameter [16]. Another concern is damage and thrombosis of surrounding vessels. It has been shown that there is minimal damage and thrombosis to surrounding vessels if the size of those vessels is greater than 3 mm [15]. The implication of these studies is that if a lesion contains a vessel greater than 3 mm, the RFA probe should be placed close to, but not in, the vessel to achieve the best outcome [15, 16]. This placement will move the tumor cells surrounding the vessel into the central ablation zone increasing the likelihood of cell death without significantly increasing damage to the vascular structure.

Other methods to mitigate the heat sink effect have also been studied. One method to overcome the heat sink effect is to occlude the blood supply to the region being ablated with a balloon catheter or gel foam [18]. Since the majority of the blood supply to HCC lesions is

http://dx.doi.org/10.5772/intechopen.72275

derived from the hepatic artery, temporary occlusion of this artery with a balloon catheter will reduce the heat sink effect and increase the size of the ablative region. Another method of occlusion is embolization of feeding vessels with gel foam. These methods enable the generation of a larger ablative zone. Risks of hepatic artery occlusion and gel foam embolization do exist and should be considered [16].

Injection of NaCl-containing solutions has also been explored to overcome the heat sink effect [16, 19–21]. Pre-treatment with hypertonic saline, 5–36%, results in significantly higher temperatures and a significantly larger ablation zone when compared to no pre-treatment [19, 20]. The NaCl solutions can be injected at any point during the procedure to expand the size of the ablation zone [21].

Transarterial chemoembolization (TACE) combined with RFA is another widely studied technique for overcoming the heat sink phenomenon. Combining these two techniques has multiple benefits that have been shown in both animal and human models [22–24]. First, TACE reduces the amount of conductive cooling by reducing blood flow to the targeted region. The reduction in blood flow increases the size of the central ablation zone, thus increasing the amount of coagulative necrosis. In addition, the amount of tissue that receives sublethal hyperthermia is increased and simultaneously exposed to a chemotherapeutic agent. The synergy is created by increased membrane permeability, intratumoral accumulation of the pharmacologic agent, and increased drug sensitivity of the cells within the transitional zone [5, 6, 8, 24]. Liposomal doxorubicin has been well studied in the setting of liver malignancies and is a good choice of chemotherapeutic agent [22, 24]. The wider ablation margin with increased volume of both the central and transitional zone and high local concentration of chemotherapeutic agent results in destruction of microscopic satellite lesions surrounding the lesion consequently improving local control of the tumor [18, 24].

2.4. Immunologic response to RFA

To adequately discuss the immunologic response to RFA, the basic immunologic response must first be discussed. The immune system is composed of two basic parts, the innate and the adaptive immune systems. Both of these systems work in concert to mount a defense to pathogens, such as viruses and bacteria, and prevent unregulated cell growth. The immune system is able to recognize and eliminate both dangerous self and non-self cells through a system of complex interactions and "danger signals." However, in HCC and other malignancies, the cells evade the immune system through numerous mechanisms [4, 25].

Typically, the first response by the immune system that occurs is by the non-specific or innate immune system. The non-specific immune system is composed of natural killer cells (NK), mast cells, eosinophils, basophils, macrophages, neutrophils, and dendritic cells (DC). These cells are the first to mobilize and produce signals initiating the specific immune response. The specific or adaptive immune system is composed of B and T lymphocytes. With co-stimulation from the innate system, the adaptive system generates a robust and lasting immune response through the formation of antibodies and memory B and T cells. The basic process that must occur to achieve a full immune response is antigen recognition and presentation

by antigen-presenting cells (APCs), subsequent recognition of the antigen by T-cells through interaction with APCs, cellular interaction generating costimulatory signals, and the presence of danger signals [4, 6]. It is important to mention the roles of CD4 or T helper cells (Th) and CD8 T cells or cytotoxic T cells (CTLs). In regards to antitumor immunity, the most important role of CD4 cells is to assist in the activation and proliferation of CD8 T cells. It is currently theorized that a high ratio of CD4:CD8 is important to forming lasting immunity to malignancies because of the role of CD4 cells in stimulating CD8 cells [30]. CD8 T cells have been the focus of antitumor immunity due to their ability to recognize MHC I molecules. MHC I molecules are used to display intracellular antigens on the surface of cells infected by viruses and malignant cells. CD8 cells bind to cells expressing specific MHC I molecule complexes and then destroy the targeted cells through the apoptotic cascade [26].

In contrast to HR where the objective is to completely remove the HCC lesion and occasionally remove local lymphoid tissues, minimally invasive techniques such as RFA leave necrosed tumor cells and their spilled intracellular materials behind. The retained intracellular materials, which were previously invisible to the immune system, can now act as antigens that trigger local and systemic immune responses to HCC. In addition to the release of antigenic material from the remaining necrotic tissues, danger signals, such as DNA, RNA, pro-inflammatory cytokines, uric acid, and heat-shock proteins (HSPs), are released. These damage-associated molecular pattern molecules (DAMPs) are then picked up by DCs and presented to T-cells starting the immune cascade. All of these DAMPs are inflammatory mediators and have the potential to trigger a robust tumor suppressing immune response but are only released by cells undergoing necrosis. Cells, which undergo apoptosis, may actually lead to tolerance if the ratio of apoptosis to necrosis within the lesion becomes too high [4, 25].

One class of DAMP of particular importance to RFA and other thermal ablative techniques is the heat shock protein (HSP) family. HSPs have special roles and are involved in protein folding, cellular signaling, cellular transport, and survival. HSPs are produced within cells in response to thermal injury and play a role as chaperones enabling the refolding of denatured proteins. Additionally, HSPs participate in the initiation of the adaptive immune response by presenting antigens to DCs, modulating DAMP-induced immune stimulation, and function as danger signals [25, 27]. HSPs' ability to chaperone peptides and provide maturation signals to dendritic cells causes the ultimate cross-presentation of antigen to CD8+ T cells. Independent of the adaptive response, HSPs induce local necrosis through stimulation of the innate immune system. HSPs' ability to efficiently stimulate both the innate and adaptive immune system holds great potential; therefore, upregulation of HSPs represents a potential approach to eliminate HCC and other malignancies [28].

In patients treated with RFA, there is a decreased response by CD25+ T-regulatory (Treg) cells [29]. Treg cells or T suppressor cells, a specific subset of T-cells, are responsible for downregulating the immune response. The Treg cell's role is to prevent autoimmune disease and create tolerance to self-antigens. Tregs achieve immunosuppression by downregulating CD4 and CD8 T cells, thus decreasing the tumoricidal immunologic response. In fact, high levels of CD25 T cells are associated with poorer outcomes in patients with malignancy [26]. Thus, patients with HCC can benefit from treatments that decrease the number of Treg cells and subsequent decreased immune tolerance of malignant cells. Indeed, RFA results in decreased

counts of suppressive T cells, but additionally RFA shows increased survival benefit due to improved CD8 T-cell counts. The post ablation increase in CD8 T-cells is strongly associated with decreased recurrence and increased survival in patients with HCC [30]. Patients who will undergo HR or liver transplantation can also benefit from RFA-induced stimulation of CD8 cells. One major issue these patients face long term is disease recurrence. Unitt et al. showed improved survival in patients who demonstrate strong CD4 and CD8 T cell responses after undergoing resection surgery [31]. The response by CD4 and CD8 T cells as well as the increase of specific antibodies has been seen weeks to months following RFA [32].

Most of our knowledge about the immune response to RFA is based on animal models and small human trials. One of the first studies to show a significant immune response to RFA was conducted in 2003. In this animal study, tumors were implanted into rabbits that were then either untouched or treated with RFA. The RFA-treated animals showed at least a threefold increase in specific T-cell infiltration compared to the untreated animals. The treated animals also showed an increase in survival rate. This study suggested that an anticancer immunologic effect could be created through RFA [33]. This study was then further augmented by blocking CTLA-4 with monoclonal antibody at the time of RFA. This strongly enhanced antitumor immunity and provided protection against tumor rechallenge. This demonstrated that a lasting systemic memory response is achievable with combination therapy. Furthermore, a 20-fold increase in specific cytotoxic T-cells (CTLs) was achieved when RFA + blocking antibody was used compared to RFA + control antibody demonstrating that the increased immune response to RFA can be potentiated [34]. Zerbini et al. were the first to demonstrate an increased immunologic response in human subjects. The effect of RFA on 20 patients with HCC was studied and found to have a significant increase in tumor-specific T-cell response. Circulating T and natural killer (NK) cells showed increased activation and expression of specific cytotoxic surface markers. Although an upregulated immune response was demonstrated in these subjects, the effect was not associated with increased protection to HCC relapse [35]. Later, Zerbini and colleagues showed that the immunologic effects post RFA are dependent on maturation of DCs driven by the release of intracellular debris [36]. In a murine urothelial carcinoma model, subtotal RFA was used to induce an immunologic response. In response to subtotal RFA, there was an increase in CD4 and CD8 responses and significant tumor regression with rechallenge [37].

It is clear that numerous benefits of RFA exist and that there is great potential for targeted stimulation of the immune system using RFA in conjunction with immune modulators. Nevertheless, the possibility of causing rapid growth of metastases exists [38–40]. Recent accounts of RFA and other forms of ablation causing growth of distant metastases have been reported. These reports in conjunction with the fact that RFA will induce mediators such as cytokines, including interleukin-6 (IL-6) and factors such as hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), hypoxia-induced factor-1α (HIF-1α), and HSPs, all have potential to cause tumor growth locally and distantly [38–42]. It is theorized that damage to the surrounding healthy liver parenchyma and following regeneration is the source of the pro-growth factors. Ahmed et al. [40] showed using a rat model that damaging normal hepatic tissue with RFA will induce distant tumor growth, which is mediated by VEGF and the HGF/c-Met pathway. Interestingly, multiple studies have shown that incomplete ablation of HCC will also promote not only distant growth but also local invasion [41, 43–45].

While increased local and distant growth of tumor cells is a real possibility, there are multiple studies investigating how to mitigate the pro-growth effects created by RFA. Studies have investigated c-Met and VEGF inhibitors to attenuate the tumorigenic effects [38, 40]. Additionally, studies have looked at non-specific anti-inflammatory drugs to mitigate the effects of RFA-induced inflammation on tumorigenesis. Both aspirin and celecoxib have been investigated to prevent tumorigenesis [41, 42]. In animal models, each of these drugs when used in conjunction with RFA reduced local inflammation and subsequent effects on distant tumor cells. Furthermore, it is crucial to note that while there is information about distant tumorigenesis following RFA, clinically RFA has not been shown to worsen survival compared to untreated patients and remains an effective first-line treatment in appropriate patients [46].

3. Cryoablation

3.1. Overview

Cryoablation is a thermal ablative technique that has been used since the nineteenth century. While other thermal ablative techniques add heat to the surrounding tissue, cryoablation removes heat. In its earliest form, a salt and ice solution was applied to breast and skin cancers. This treatment resulted in decreased pain and lesion size. In its current form, cryoablation is performed similarly to other ablative techniques. It can be used in either a percutaneous fashion, with image guidance, or through an open or laparoscopic surgical approach. Cryoablation is used to treat numerous types of cancers, but is most commonly used to treat liver, kidney, lung, prostate, and breast malignancies [47].

Modern cryoablation requires the use of a specialized cryoprobe that is inserted into the targeted lesion (**Figure 1**). Once in the desired location, the probe is rapidly cooled beginning the freeze cycle for a specified length of time. After the freeze cycle is completed, the probe is warmed up to start the thaw cycle. These freeze/thaw cycles are repeated one or more times depending on the lesion and preference of the clinician [47]. The mechanism of cooling relies on the Joule-Thompson effect that describes how a gas that does not work expands (adiabatic expansion) and results in a decrease in temperature [48]. All gases except hydrogen, helium, and neon will decrease in temperature when expanded through the Joule-Thompson process. Commonly used gases for the freeze cycle are nitrogen and argon. One of these gases is pumped into the cryoprobe, and when the gas reaches the distal tip of the probe, the gas is throttled and then allowed to rapidly expand to atmospheric pressure. The result is a rapid decrease in temperature and cooling of the surrounding tissues via conduction. During the freeze cycles, temperatures can reach as low as −160°C, well below the −20 to −40°C required to cause cell death [49]. As mentioned above, helium does not undergo this effect, rather than cooling when rapidly expanded helium will increase the temperature. For this reason, helium is used in the thaw cycle to heat the surrounding tissues [47].

Cryoablation results in direct and indirect cellular injury and death. When the freeze cycle begins, the tissues are cooled and ice starts to form in the extracellular space. Since the formation of ice occurs in the extracellular space before the intracellular space, an osmotic gradient

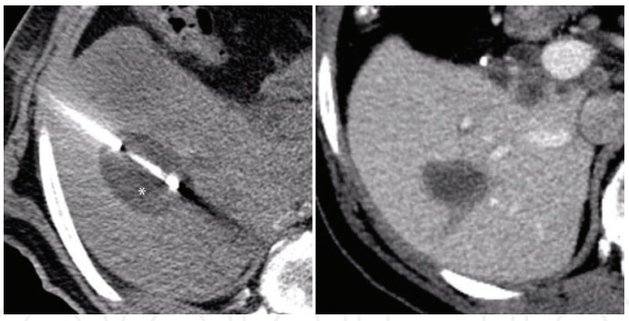

Figure 1. Hepatic cryoablation. A cryoablation needle was advanced into a focal hepatic lesion (left), with a resultant ice ball visible on CT (asterisk). Follow-up imaging demonstrated a focal defect at the site of the previous lesion (right), consistent with a complete ablation.

forms. This gradient pulls free water into the extracellular space increasing the solute concentration and dehydrating the cells. The high concentration of solutes intracellularly results in damage to enzymes and destabilizes membranes of both intracellular organelles and the cell [47]. Intracellular proteins are denatured as well, but return to their original conformation once thawing is completed [50]. Cells at the periphery of the ablation will remain intact and are not immediately killed by cryoablation. These cells will eventually undergo apoptosis that is triggered by damage to organelles [47].

When cells are frozen rapidly, there is no enough time for fluid shifts to occur, and cell death occurs via physical damage to organelles and the cellular membrane from intracellular ice formation. In both cases, pore formation in the membrane occurs, which allows for fluid shifts during the thaw cycle resulting in swelling and rupture [51]. The intracellular fluid shift during the thaw cycle occurs since extracellular ice melts before intracellular ice creating an osmotic gradient into the cell. It is important to note that intracellular ice will continue to grow during the thaw cycle reaching a maximum at −20 to −25°C. This formation of ice during the thaw cycle occurs due to the influx of free water. Additionally, the rate of thawing determines the amount of cellular death. Rapid thawing will decrease the biocidal effect by reducing the amount of intracellular ice formation. A greater degree of cellular death is seen in passive thawing when compared to active thawing. The highest degree of necrosis is seen with repeated freeze thaw cycles [52].

Indirect injury to cells occurs via vascular damage. During the freeze cycle, the endothelium of vessels is damaged, and when thawed, this injury triggers platelet aggregation. This aggregation leads to thrombosis and subsequent ischemia of the tissues [53]. The ischemia is twofold, not only does it lead to cellular death, but it also triggers inflammation. This leads to an influx of neutrophils and macrophages to the ablated zone [54]. The entire process can take months to complete, resulting in a zone of necrosis surrounded by a peripheral band of neutrophils [47].

3.2. Advantages and disadvantages of cryoablation

The one of the best advantages of cryoablation is the ability to monitor the ablation zone in real time. As the ablation proceeds, formation of an ice-ball occurs that is visible on ultrasound (US), magnetic resonance (MR), and CT. This occurs because the water molecules undergo a

phase change and subsequent change in density. For example, during a cryoablation, the ablative zone will become hypoattenuating on CT. The leading edge of the ablation marks 0°C, since this region is where the phase change from liquid to solid is occurring [47].

In contrast to other modalities, such as RFA, each cryoprobe acts independently from the others and can be used simultaneously to tailor the shape of the ablation to the tumor. This is in sharp contrast to ablating with multiple RF probes where only one probe can be active at a time and they must be operated sequentially. Cryoablation additionally offers better pain control when compared to RFA [47]. The cooling of the tissues can create a level of analgesia not offered by hyperthermic ablative techniques. Cryoablation significantly reduces the amount of opioids used in the 24 hours following the procedure leading to shorter hospital stays [55]. In regards to HCC, it has been shown that RFA can induce ischemia-reperfusion injury of the liver resulting in cancer growth. With cryoablation, there is a lower potential for this type of injury decreasing the risk of cancer growth [56]. Cryoablation can also be used in patients who are candidates for RFA. Patients who are candidates are those with tumors <5 cm, single lesions, or multiple lesions <3 cm with a Child-Pugh class A or B liver function [57].

Cryoablation results in a robust inflammatory response following the procedure. This in combination with the fact that the released proteins return to their native conformation produces a large potential to create beneficial antitumor immunologic responses. The large amount of unaltered tumor antigen coupled with a large inflammatory response creates a scenario in which significant numbers of DCs are able to present a large amount of antigen to T cells [47, 50, 54]. The potential immunologic response will be discussed in detail later. This robust inflammatory response also presents a significant disadvantage, cryoshock [47, 57–59].

Cryoshock is a systemic immune response that leads to hypotension, respiratory distress, multiorgan failure, and disseminated intravascular coagulation. Similar reactions are not seen in patients treated with hyperthermic ablations. Cryoshock occurs in up to 1% of patients who undergo hepatic cryotherapy. Of this 1%, up to 18% of patients can die because of cryoshock [57, 58]. Cryoshock is thought to be mediated by the production of cytokine, such as IL-1β, IL-6, and tumor necrosis factor (TNF), from the robust immune response created by cryoablation [47, 57–60]. These are similar to the mediators found in patients with septic shock [59, 65]. Cryoshock typically occurs when large volume liver ablations are attempted [47]. An additional disadvantage of cryoablation is bleeding complications. Typically, these occur when performing large ablations within the liver. Frozen tissues are extremely brittle and may fracture leading to significant bleeding. For this reason, the user must be careful to not torque or reposition the probes once the ablation has started [61]. Although cryoablation has various disadvantages, it has a similar complication rate compared to RFA and remains a relatively safe and effective procedure for the treatment of HCC [57–60, 62].

3.3. Immunologic response to cryoablation

For several decades, the immune response to cryoablation has been known. In the 1970s, antitumor antibodies were first seen in humans following cryoablation [63]. Since then, it has been shown that cryoablation will induce specific anti-tumor cytotoxic effects post ablation. Lymphocytes produced post-ablation show specific affinity for tumor cells when rechallenged,

whereas lymphocytes in patients post HR do not. Reintroduced tumor cells are also rendered less effective by the immunologic response [64].

Cryoablation is proven to create a more robust immunological response than hyperthermic ablative techniques such as RFA and MWA. Higher DC antigen loading due to hypothermic cytotoxicity generates the more robust immunologic response. Increased antigen presentation and subsequent heightened immune response are due to two main factors: increased antigen in the native conformation and less coagulation in the ablation zone [47]. The hypothermic mechanism of cryoablation leads to less regional coagulative effect when compared to hyperthermic ablations. This allows the antigens produced by cryoablation to more readily enter circulation and regional lymph nodes for presentation to DCs. The ability of cryoablation to preserve circulation is beneficial for stimulating the immune system but can be detrimental. The spilling of tumor antigens into circulation and subsequent cytokine release is also believed to be responsible for cryoshock.

Heightened levels of cytokines such as IL-1β, IL-6, TNF-α, and NF-κB are seen after cryoablation [65, 66]. Cryoablation used on hepatic malignancy will increase the levels of these specific cytokines up to 15–25 times more when compared to RFA. Interestingly, Erinjeri et al. found that the changes in WBC count increased linearly with ablation size but the levels of IL-6 did not [65], suggesting that larger ablative zones could trigger higher tumor-specific immune responses without added increased risk for cryoshock. Additionally, this group found that the predominant cytokines that are released post cryoablation, IL-6 and IL-10, stimulate a Th2 response.

According to the Th1/Th2 model, each subset triggers a different type of immunity. Th1 triggers cytotoxic lymphocytes and cellular immunity, whereas Th2 stimulates B-cells and antibody production. Signals that stimulate the Th1 response include IL-2, IL-12, IFN-γ, and TNF, and the cytokines that trigger the Th2 response include IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 [65, 67]. In addition, IL-6, which activates NF-κB and STAT3, has been implicated in hepatic regeneration and increased tumor regeneration post ablation. Regeneration is mediated by NF-κb and STAT3, both of which are activated by IL-6 [65]. Since, both IL-6 and IL-10 are increased after not only cryoablation but also hyperthermic ablation, they are potential targets for adjuvant immunologic therapy. Decreasing IL-6 could potentially increase the Th1 response (cellular-mediated tumor immunity) and decrease the growth of primary and metastatic hepatic malignancy post ablation [68].

Immune checkpoint inhibitor therapy, a new oncologic therapy that uses monoclonal antibody to target and block the T cell surface receptor CTLA-4, has potential use in combination therapy with cryoablation. The function of CTLA-4 is to inhibit self-reactive T-cells in order to prevent autoimmune diseases. However, in the case of cancer, it is beneficial for T cells to be able to recognize specific "self" cells. It has been shown that blockade of CTLA-4 will increase the CD8 T cell response as well as CD4 T cell memory when used as a monotherapy or combination immune therapy [69, 70]. Phase 3 trials using ipilimumab, a CTLA-4 blocking monoclonal antibody, have showed improved recurrence-free survival when used as an adjuvant treatment in patients with high-risk melanoma [71]. Combination cryotherapy with CTLA-4 blockade has been studied in prostate cancer. While cryoablation alone has not been

shown to mediate the rejection of metastatic lesions, when combined with CTLA-4 blockade, it can mediate rejection of metastatic lesions and prevent disease recurrence [72].

While cryoablation has been strongly shown to activate the immune system, the opposite has also been seen. Multiple animal models have shown susceptibility to rechallenge and increased metastasis post ablation [73–75]. A possible explanation for these results is variation in the technical factors of cryoablation. Differing animal models, methods of freezing, length, number of freeze-thaw cycles, differences in minimum temperature achieved as well as differing ablation zone size and position all contribute to different clinical outcome and immunologic stimulation or anergy [73]. Sabel et al. established that variation in the technical parameters of cryoablation indeed affect the ratio of apoptosis to necrosis and subsequent immune response. Sabel et al. investigated the rate of freezing in an animal model using either a low or high rate of freezing. They found that a high rate of freezing induced a higher amount of necrosis when compared to a low rate of freezing. The high rate induced more danger signals stimulating a strong anti-tumor response [73]. A high ratio of apoptosis to necrosis has been shown to downregulate the immunologic response and even induce anergy [76]. When apoptotic cells are presented to DCs, a lower amount of TNF-α, IL-1β, IL-8, IL-10, IL-12, and granulocyte macrophage colony-stimulating factor (GM-CSF) produce inhibitory effects on these cells [77, 78]. The ablation zone size and percentage of tumor encompassed may play a role in the immunologic response. An experiment conducted in a murine metastatic liver tumor model demonstrated that smaller volume ablations show a significant decrease in metastasis [79].

4. Microwave ablation

4.1. Overview

Microwave ablation (MWA) is a hyperthermic ablative technique that is similar in many ways to RFA. MWA was introduced in the 1980s and 1990s and showed potential, but suffered from problems controlling the emitted field. There was a relatively high complication rate with MWA, thus RFA became the dominant ablative technique [80, 81]. While the early MWA systems had higher complication rates, since then newer designs have significantly decreased the complication rates. Recent retrospective and prospective studies have proven the efficacy and safety of MWA for not only HCC lesions but also other hepatic lesions [82–85].

From a procedural point of view, MWA and RFA are performed similarly under image guidance. The operator guides a MWA antenna toward the targeted lesion using their favored imaging modality (**Figure 2**). Unlike RFA, MWA does not require the use of grounding pads to establish an electrical circuit. MWA uses dielectric hysteresis to produce heat. An oscillating field, typically 900–2500 MHZ, is applied forcing polar molecules (such as water) to continuously move and realign in the field creating kinetic energy and ultimately raising the temperature of the tissue. Microwaves are able to propagate through a variety of tissues, even those with low electrical conductivity, high impedance, or low thermal conductivity. This makes MWA more versatile [86].

http://dx.doi.org/10.5772/intechopen.72275

4.2. Advantages and disadvantages of MWA

MWA has the distinct advantage of being able to penetrate through high impedance tissues, meaning that even if charred or desiccated tissues build up near the probe, the field is able to penetrate and continue enlarging the ablation zone. Since MWA does not rely on conduction of tissue, heat is able to penetrate tissues with a high impedance such as lung or bone [86].

Multiple MWA antennas are able to be used synergistically to enlarge the ablation zone, achieve higher temperatures, or concomitantly ablate multiple lesions [87]. While RFA using multiple probes requires the probes to be used in series, MWA with multiple antennas can be used simultaneously with one power source. Due to the properties of MWA, there is future potential use ablating larger lesions than is currently possible.

The peak temperatures achieved in the central zone can readily exceed 100°C. The ability to achieve higher temperatures and use multiple probes simultaneously means shorter treatment times and larger area of coagulative necrosis and lethal hyperthermia. Higher temperatures and larger central zone lessen the effect of nearby heat sinks. It has been shown that large vessels <10 mm in size will not affect the ablation, making it possible to ablate lesions in regions that are not possible with RFA [88].

While MWA has many advantages, its ability to deliver a high amount of energy comes with several trade-offs. Coaxial cables have excellent properties for this application and are thus used to connect the antenna to the microwave generator. However, the coaxial cables used have a large diameter in order to avoid dangerous cable overheating. Larger diameter decreases the risk of overheating but becomes cumbersome and inflexible leading to difficulties while manipulating the antennas and performing the procedure [89]. The microwave antennas are likewise made using coaxial cable and also suffer from the same problem. In order for the antenna to handle higher power levels, the diameter must be increased or an active cooling system needs to be employed [86, 89].

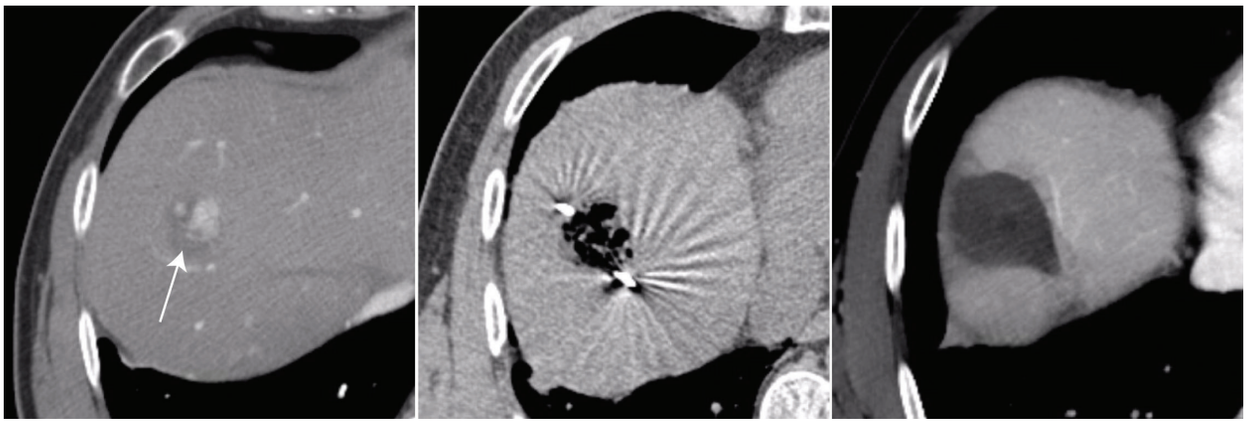

Figure 2. Hepatic microwave ablation. Pre-procedure imaging demonstrates a focal HCC lesion at the hepatic dome (left, arrow). Two microwave needles were advanced into the lesion, with gas bubbles developing during the ablation (middle). Follow-up imaging demonstrates a focal defect without enhancing viable tissue consistent with a complete ablation (right).

Active cooling systems have helped eliminate several problems. They allow a smaller diameter antenna to handle higher power and eliminate the risk of ablating healthy tissues along the proximal antenna tract, increase the size of the ablation, increase the amount of power delivered, and can prevent the probe from backing out. Various cooling methods have been employed from chilled saline to cooling with compressed gas utilizing the Joule-Thomson phenomenon [86]. Some newer probes that use the Joule-Thompson phenomenon are able to be locked into place by freezing the antenna tract to prevent it from moving or backing out.

Since the primary advantage of MWA is the ability to deliver, a significant amount of power safety concerns arises. With MWA, it is harder to predict the size of the ablation zone, which can lead to damage of surrounding structures. The shape of the ablation zone produced can be relatively thin and long increasing this risk. While problems with MWA exist, currently these issues should not limit its use ablating HCC and other lesions in the liver. MWA has been proven effective and comparatively safe to RFA when measuring complication rates [82–85].

4.3. Immunologic response to MWA

The immunologic response to MWA is less well characterized compared to other methods of ablation, such as RFA and cryoablation. The vast majority of the research regarding the immune response and immunologic stimulation has been studied with either RFA or cryoablation. Recently specific immunologic mechanisms and the effects following MWA have been studied in more detail. It has been assumed that the immunologic response to MWA is similar to the mechanism and response to RFA [90, 91]. Currently, our knowledge about the immune response to MWA comes from both animal and clinical trials of various tumor types from breast to hepatic carcinomas [92–94].

In regards to patient management and future treatment, the goal of therapy is to generate a lasting immune response that results in regression of distant lesions and generate protection from disease recurrence. As detailed in previous sections, the aim of treatment is to generate specific cytokines triggering the Th1 response and activating the cellular immune system. Similar to other ablative techniques, MWA alone is not powerful enough to trigger the desired immune response, but holds potential with combination therapy [90, 91].

The specific immune response to MWA in patients with HCC has been analyzed by Zhang et al. In their study, 45 patients with HCC treated with MWA had peripheral blood analysis following treatment. The results showed significant increases in IL-12 and decreases in IL-4 and IL-10 [91]. These results are promising since IL-12 is involved in the differentiation of Th1 cells and generation of cellular immunity. Furthermore, patients showed decreased levels of IL-4 and IL-10, which are involved in activation of humoral immunity. While MWA alone is not enough to create a significantly different clinical response, the cytokine profile produced is advantageous.

Various combination therapies have been studied ranging from OK-432, immunotherapy, GM-CSF, and CTLA-4 blockade [92–94]. OK-432, also known as picibanil, is a low virulence mixture of *Streptococcus pyogenes* that has been used as an antitumor agent since 1975 [95].

http://dx.doi.org/10.5772/intechopen.72275

OK-432 is able to induce pro-inflammatory cytokines and activate the T-cell–mediated immunity. Li et al. demonstrated that MWA and OK-432 used in combination resulted in prolonged survival and a strong immunologic response to rechallenge in a murine model of breast cancer. The results showed that a dominant Th1 response is generated. The cytokines IL-12, IL-2, and IFN-γ were significantly increased with no effect on Th2-type cytokines. Additionally, immunohistochemical analysis showed that a predominance of CD8+ T cells infiltrating the treated tumors.

Immunotherapy combined with MWA for the treatment of HCC has been investigated in both phase I and phase II trials [93, 96]. This combination was shown to increase the absolute number of circulating lymphocytes. When analyzed for specific subgroups, patients showed increased levels of cytotoxic subsets of T cells and decreased suppressive subsets. Additionally, patients treated with immunotherapy had significantly improved liver function. However, the disease-free survival and overall survival rate were not significantly improved.

CTLA-4 blockade holds great promise when combined with cryoablation, but could also be used in with MWA. CTLA-4 blocking antibodies and GM-CSF combined with MWA were shown to induce tumor-specific cellular immune response in a murine model [94]. The combination of the three resulted in a 90% rejection upon tumor rechallenge and 50% of the animals treated showed distant tumor regression. Since both of these drugs are currently available for human use, this combination represents one that could be clinically used today.

5. Conclusion

Minimally invasive thermal-based therapy has become a reliable method for the treatment of HCC. Many advances in ablative therapy have occurred since their initial implementation ranging from design to technical implementation. The most promising of these advances is combination therapies that create a tumor-specific immunologic response. Combination therapy has shown great promise in the treatment and prevention of not only HCC, but also other malignancies. There is much more to learn about the immunologic reaction to ablative therapy creating an exciting time of investigation and discovery.

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References

[1] Li G, Staveley-O'Carroll K, Kimchi E. Potential of radiofrequency ablation in combination with immunotherapy in the treatment of hepatocellular carcinoma. Journal of Clinical Trials. Apr 2016;**6**(2):1-9. DOI: 10.4172/2167-0870.1000257

[2] Cui J, Wang N, Zhao H, Haofan J, Wang G, Niu C, Terunuma H, He H, Li W. Combination of radiofrequency ablation and sequential cellular immunotherapy improves progression-free survival for patients with hepatocellular carcinoma. International Journal of Cancer. 2014;**134**:342-351. DOI: 10.1002/ijc.28372

[3] Takaki H et al. Thermal ablation and immunomodulation: From preclinical experiments to clinical trials. Diagnostic and Interventional Imaging. 2017;**98**:651-659

[4] Bastianpillai C, Petrides N, Shah T, Guillaumier S, Ahmed H, Arya M. Harnessing the immunomodulatory effect of thermal and non-thermal ablative therapies for cancer treatment. Tumour Biology. Dec 2015;**36**(12):9137-9146. DOI: 10.1007/s13277-015-4126-3

[5] Chu K, Dupuy D. Thermal ablation of tumours: Biological mechanisms and advances in therapy. Nature Reviews Cancer. Mar 2014;**14**(3):199-208. DOI: 10.1038/nrc3672

[6] Mehta A, Oklu R, Sheth R. Thermal ablative therapies and immune checkpoint modulation: Can locoregional approaches effect a systemic response? Gastroenterology Research and Practice. 2016;**2016**:9251375. DOI: 10.1155/2016/9251375

[7] Goldberg SN, Ahmed M, Gazelle GS, Kruskal JB, Huertas JC, Halpern EF, Oliver BS, Lenkinski RE. Radio-frequency thermal ablation with NaCl solution injection: Effect of electrical conductivity on tissue heating and coagulation-phantom and porcine liver study. Radiology. 2001;**219**(1):157-165. DOI: 10.1148/radiology.219.1.r01ap27157

[8] Ahmed M, Brace C, Lee F, Goldberg S. Principles of and advances in percutaneous ablation. Radiology. 2011;**258**(2):351-369. DOI: 10.1148/radiol.10081634

[9] Thompson SM, Callstrom MR, Butters KA, Knudsen B, Grande JP, Roberts LR, Woodrum DA. Heat stress induced cell death mechanisms in hepatocytes and hepatocellular carcinoma: In vitro and in vivo study. Lasers in Surgery and Medicine. 2014; **46**(4):290-301. DOI: 10.1002/lsm.2223

[10] Duan C, Liu M, Zhang Z, Ma K, Bie P. Radiofrequency ablation versus hepatic resection for the treatment of early-stage hepatocellular carcinoma meeting Milan criteria: A systematic review and meta-analysis. World Journal of Surgical Oncology. 2013;**11**(1):190. DOI: 10.1186/1477-7819-11-190

[11] DS L, NC Y, Raman SS, Limanond P, Lassman C, et al. Radiofrequency ablation of hepatocellular carcinoma: Treatment success as defined by histologic examination of the explanted liver. Radiology. 2005;**234**(3):954-960. DOI: 10.1148/radiol.2343040153

[12] Dromain C, de Baere T, Elias D, et al. Hepatic tumors treated with percutaneous radiofrequency ablation: CT and MR imaging follow-up. Radiology. 2002;**223**(1):255-262. DOI: 10.1148/radiol.2231010780

[13] Molinari M, Helton S. Hepatic resection versus radiofrequency ablation for hepatocellular carcinoma in cirrhotic individuals not candidates for liver transplantation: A Markov model decision analysis. American Journal of Surgery. 2009;**198**(3):396-406. DOI: 10.1016/j.amjsurg.2009.01.016

[14] Cho YK, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: A Markov model analysis. Hepatology. 2010;**51**(4):1284-1290. DOI: 10.1002/hep.23466

[15] DS L, Raman SS, Vodopich DJ, Wang M, Sayre J, Lassman C. Effect of vessel size on creation of hepatic radiofrequency lesions in pigs: Assessment of the "heat sink" effect. American Journal of Roentgenology. 2002;**178**(1):47-51. DOI: 10.2214/ajr.178.1.1780047

[16] DS L, Raman SS, Limanond P, Aziz D, Economou J, Busuttil R, Sayre J. Influence of large peritumoral vessels on outcome of radiofrequency ablation of liver tumors. Journal of Vascular and Interventional Radiology. 2003;**14**(10):1267-1274

[17] Rossi S, Garbagnati F, De Francesco I, Accocella F, Leonardi L, Quaretti P, Zangrandi A, Paties C, Lencioni R. Relationship between the shape and size of radiofrequency induced thermal lesions and hepatic vascularization. Tumori. 1999;**85**(2):128-132

[18] Rossi S, Garbagnati F, Lencioni R, Allgaier HP, Marchianò A, Fornari F, Quaretti P, Tolla GD, Ambrosi C, Mazzaferro V, Blum HE, Bartolozzi C. Percutaneous radio-frequency thermal ablation of nonresectable hepatocellular carcinoma after occlusion of tumor blood supply. Radiology. 2000;**217**(1):119-126. DOI: 10.1148/radiology.217.1.r00se02119

[19] Ahmed M, Lobo SM, Weinstein J, Kruskal JB, Gazelle GS, Halpern EF, Afzal SK, Lenkinski RE, Goldberg SN. Improved coagulation with saline solution pretreatment during radiofrequency tumor ablation in a canine model. Journal of Vascular and Interventional Radiology. 2002;**13**(7):717-724

[20] Lee JM, Han JK, Kim SH, Shin KS, Lee JY, Park HS, Hur H, Choi BI. Comparison of wet radiofrequency ablation with dry radiofrequency ablation and radiofrequency ablation using hypertonic saline preinjection: Ex vivo bovine liver. Korean Journal of Radiology. 2004;**5**(4):258-265. DOI: 10.3348/kjr.2004.5.4.258

[21] A1 S, Ishizaka H, Awata S, Shiraishi A, Hirasawa S, Tatezawa T, Kano M, Shimodaira K, Taketomi-Takahashi A, Tsushima Y, Endo K. Expansion of radiofrequency ablation volume by saturated NaCl saline injection in the area of vaporization. Acta Radiologica. 2009;**50**(1):61-64. DOI: 10.1080/02841850802562071

[22] Ahmed M, Goldberg SN. Combination radiofrequency thermal ablation and adjuvant IV liposomal doxorubicin increases tissue coagulation and intratumoural drug accumulation. International Journal of Hyperthermia. 2004;**20**(7):781-802

[23] Goldberg SN, Kamel IR, Kruskal JB, Reynolds K, Monsky WL, Stuart KE, Ahmed M, Raptopoulos V. Radiofrequency ablation of hepatic tumors: Increased tumor destruction with adjuvant liposomal doxorubicin therapy. AJR – American Journal of Roentgenology. 2002;**179**(1):93-101. DOI: 10.2214/ajr.179.1.1790093

[24] Higgins M, Soulen M. Combining locoregional therapies in the treatment of hepatocellular carcinoma. Seminars in Interventional Radiology. 2013;**30**(1):74-81. DOI: 10.1055/s-0033-1333656

[25] Pradeu T, Cooper EL. The danger theory: 20 years later. Frontiers in Immunology. 2015; **3**(287):56-61

[26] Adeegbe DO, Nishikawa H. Natural and induced T regulatory cells in cancer. Frontiers in Immunology. 2013;**4**:190. DOI: https://doi.org/10.3389/fimmu.2013.00190

[27] Calderwood SK, Ciocca DR. Heat shock proteins: Stress proteins with Janus-like properties in cancer. International Journal of Hyperthermia. 2008;**24**(1):31-39

[28] Milani V, Noessner E, Ghose S, Kuppner M, Ahrens B, Scharner A, Gastpar R, Issels RD. Heat shock protein 70: Role in antigen presentation and immune stimulation. International Journal of Hyperthermia. 2002;**18**(6):563-575. DOI: 10.1080/02656730210166140

[29] Fietta AM, Morosini M, Passadore I, Cascina A, Draghi P, Dore R, Rossi S, Pozzi E, Meloni F. Systemic inflammatory response and downmodulation of peripheral CD25+Foxp3+ T-regulatory cells in patients undergoing radiofrequency thermal ablation for lung cancer. Human Immunology. 2009;**70**(7):477-486. DOI: 10.1016/j.humimm.2009.03.012 Epub 2009 Mar 27

[30] Hiroishi K, Eguchi J, Baba T, Shimazaki T, Ishii S, Hiraide A, Sakaki M, Doi H, Uozumi S, Omori R, Matsumura T, Yanagawa T, Ito T, Imawari M. Strong CD8(+) T-cell responses against tumor-associated antigens prolong the recurrence-free interval after tumor treatment in patients with hepatocellular carcinoma. Journal of Gastroenterology. 2010; **45**(4):451-458. DOI: 10.1007/s00535-009-0155-2

[31] Unitt E, Marshall A, Gelson W, Rushbrook SM, Davies S, Vowler SL, Morris LS, Coleman N, Alexander GJ. Tumour lymphocytic infiltrate and recurrence of hepatocellular carcinoma following liver transplantation. Journal of Hepatology. 2006;**45**(2):246-253. DOI: 10.1016/j.jhep.2005.12.027

[32] Widenmeyer M, Shebzukhov Y, Haen SP, Schmidt D, Clasen S, Boss A, Kuprash DV, Nedospasov SA, Stenzl A, Aebert H, Wernet D, Stevanović S, Pereira PL, Rammensee HG, Gouttefangeas C. Analysis of tumor antigen-specific T cells and antibodies in cancer patients treated with radiofrequency ablation. International Journal of Cancer. 2011; **128**(11):2653-2662. DOI: 10.1002/ijc.25601

[33] Wissniowski TT, Hänsler J, Neureiter D, Frieser M, Schaber S, Esslinger B, Voll R, Strobel D, Hahn EG, Schuppan D. Activation of tumor-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits. Cancer Research. 2003;**63**(19):6496-6500

[34] Den Brok MH, Sutmuller RP, van der Voort R, Bennink EJ, Figdor CG, Ruers TJ, et al. In situ tumor ablation creates an antigen source for the generation of antitumor immunity. Cancer Research. 2004;**64**(11):4024-4029

[35] Zerbini A, Pilli M, Penna A, Pelosi G, Schianchi C, Molinari A, et al. Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. Cancer Research. 2006;**66**(2):1139-1146

[36] Zerbini A, Pilli M, Fagnoni F, Pelosi G, Pizzi MG, Schivazappa S, et al. Increased immunostimulatory activity conferred to antigen-presenting cells by exposure to antigen extract from hepatocellular carcinoma after radiofrequency thermal ablation. Journal of Immunotherapy. 2008;**31**(3):271-282. DOI: 10.1097/CJI.0b013e318160ff1c

[37] Dromi SA, Walsh MP, Herby S, Traughber B, Xie J, Sharma KV, et al. Radiofrequency ablation induces antigen-presenting cell infiltration and amplification of weak tumor-induced immunity. Radiology. 2009;**251**(1):58-66

[38] Rozenblum N, Zeira E, Scaiewicz V, Bulvik B, Gourevitch S, Yotvat H, Galun E, Goldberg SN. Oncogenesis: An "off-target" effect of radiofrequency ablation. Radiology. 2015;**276**(2):426-432. DOI: 10.1148/radiol.2015141695

[39] Nijkamp M, Borren A, Govaert K, et al. Radiofrequency ablation of colorectal liver metastases induces an inflammatory response in distant hepatic metastases but not in local accelerated outgrowth. Journal of Surgical Oncology. 2010;**101**(7):551-556

[40] Ahmed M, Kumar G, Moussa M, et al. Hepatic radiofrequency ablation-induced stimulation of distant tumor growth is suppressed by c-Met inhibition. Radiology. 2016;**279**(1):103-117. DOI: 10.1148/radiol.2015150080

[41] Jiang T, Zhang X, Ding J, Duan B, Inflammation LS. Cancer: Inhibiting the progression of residual hepatic VX2 carcinoma by anti-inflammatory drug after incomplete radiofrequency ablation. International Journal of Clinical and Experimental Pathology. 2015;**8**(11):13945-13956

[42] Kumar G, Goldberg SN, Wang Y, Velez E, Gourevitch S, Galun E, Ahmed M. Hepatic radiofrequency ablation: Markedly reduced systemic effects by modulating periablational inflammation via cyclooxygenase-2 inhibition. European Radiology. 2017;**27**(3):1238-1247. DOI: 10.1007/s00330-016-4405-4

[43] Zhang N, Wang L, Chai ZT, Zhu ZM, Zhu XD, Ma DN, Zhang QB, Zhao YM, Wang M, Ao JY, Ren ZG, Gao DM, Sun HC, Tang ZY. Incomplete radiofrequency ablation enhances invasiveness and metastasis of residual cancer of hepatocellular carcinoma cell HCCLM3 via activating β-catenin signaling. PLoS One. 2014;**9**(11):e115949. DOI: 10.1371/journal.pone.0115949

[44] Kong J, Kong L, Ke S, Gao J, Ding X, Zheng L, Sun H, Sun W. After insufficient radiofrequency ablation, tumor-associated endothelial cells exhibit enhanced angiogenesis and promote invasiveness of residual hepatocellular carcinoma. Journal of Translational Medicine. 2012;**10**:230. DOI: 10.1186/1479-5876-10-230

[45] Kong J, Pan B, Ke S, Dong S, Li X, Zhou A, Zheng L, Sun WB. Insufficient radiofrequency ablation promotes angiogenesis of residual hepatocellular carcinoma via HIF-1alpha/VEGFA. PLoS One. 2012;**7**:e37266. DOI: 10.1371/journal.pone.0037266

[46] Lencioni R, Cioni D, Crocetti L, et al. Early-stage hepatocellular carcinoma in patient with cirrhosis: Long-term results of percutaneous image-guided radiofrequency ablation. Radiology. 2005;**234**(3):961-967. DOI: 10.1148/radiol.2343040350

[47] Erinjeri JP, Clark TW. Cryoablation: Mechanism of action and devices. Journal of Vascular and Interventional Radiology. 2010;**21**(8):187-191. DOI: 10.1016/j.jvir.2009.12.403

[48] O'Rourke AP, Haemmerich D, Prakash P, Converse MC, Mahvi DM, Webster JG. Current status of liver tumor ablation devices. Expert Review of Medical Devices . 2014;**4**(4):523-537. DOI: http://dx.doi.org/10.1586/17434440.4.4.523

[49] Baust J, Gage A, Ma H, Zhang CM. Minimally invasive cryosurgery—Technological advances. Cryobiology. 1997;**34**(4):373-384. DOI: https://doi.org/10.1006/cryo.1997.2017

[50] Privalov P. Cold denaturation of protein. Critical Reviews in Biochemistry and Molecular Biology. 1990;**25**(4):281-306. DOI: http://dx.doi.org/10.3109/10409239009090612

[51] Baust J, Gage A. The molecular basis of cryosurgery. BJU International. 2005;**95**(9):1187-1191. DOI: 10.1111/j.1464-410X.2005.05502.x

[52] Woolley M, Schulsinger D, Durand D, Zeltser I, Waltzer W. Effect of freezing parameters (freeze cycle and thaw process) on tissue destruction following renal Cryoablation. Journal of Endourology. 2004;**16**(7):519-522. DOI: https://doi.org/10.1089/089277902760367494

[53] Finelli A, Rewcastle J, Jewett M. Cryotherapy and radiofrequency ablation: Pathophysiologic basis and laboratory studies. Current Opinion in Urology. 2003;**13**(3):187-191

[54] Weber S, Lee F, Chinn D, Warner T, Chosy S, Mahvi D. Perivascular and intralesional tissue necrosis after hepatic cryoablation: Results in a porcine model. Surgery. 1997;**122**(4):742-747. DOI: https://doi.org/10.1016/S0039-6060(97)90082-9

[55] Thacker PG, Callstrom MR, Curry TB, Mandrekar JN, Atwell TD, Goetz MP, Rubin J. Palliation of painful metastatic disease involving bone with imaging-guided treatment: Comparison of patients' immediate response to radiofrequency ablation and cryoablation. AJR – American Journal of Roentgenology. 2011;**197**(2):510-515. DOI: 10.2214/AJR.10.6029

[56] Song K. Percutaneous cryoablation for hepatocellular carcinoma. Clinical and Molecular Hepatology. 2016;**22**(4):509-515. DOI: 10.3350/cmh.2016.0079

[57] Yang Y, Wang C, Lu Y, Bai W, An L, Qu J, Gao X, Chen Y, Zhou L, Wu Y, Feng Y, Zhang M, Chang X, Ly J. Outcomes of ultrasound-guided percutaneous argon-helium cryoablation of hepatocellular carcinoma. Journal of Hepato-Biliary-Pancreatic Sciences. 2012;**19**(6):674-684. DOI: 10.1007/s00534-011-0490-6

[58] Seifert JK, Morris DL. World survey on the complications of hepatic and prostate cryotherapy. World Journal of Surgery. 1999;**23**(2):109-113

[59] Seifert JK, Stewart GJ, Hewitt PM, Bolton EL, Junginger T, Morris DL. Interleukin-6 and tumor necrosis factor-α levels following hepatic Cryotherapy: Association with volume and duration of freezing. World Journal of Surgery. 1999;**23**(10):1019-1026

[60] Sheen AJ, Poston GJ, Sherlock DJ. Cryotherapeutic ablation of liver tumours. The British Journal of Surgery. 2002;**89**(11):1396-1401. DOI: 10.1046/j.1365-2168.2002.02292.x

[61] Hruby G, Edelstein A, Karpf J, et al. Risk factors associated with renal parenchymal fracture during laparoscopic cryoablation. BJU International. 2008;**102**(6):723-726. DOI: 10.1111/j.1464-410X.2008.07735.x

[62] Adam R, Hagopian EJ, Linhares M, Krissat J, Savier E, Azoulay D, Kunstlinger F, Castaing D, Bismuth H. A comparison of percutaneous cryosurgery and percutaneous radiofrequency for unresectable hepatic malignancies. Archives of Surgery. 2002;**137**(12):1332-1339

[63] Soanes WA, Ablin RJ, Gonder MJ. Remission of metastatic lesions following cryosurgery in prostatic cancer: Immunologic considerations. The Journal of Urology. 1970;**104**(1): 154-159

[64] Neel HB, Ketcham AS, Hammond WG. Experimental evaluation of in situ oncocide for primary tumor therapy: Comparison of tumor-specific immunity after complete excision, cryonecrosis and ligation. The Laryngoscope. 1973;**83**(3):376-387. DOI: 10.1288/00005537-197303000-00009

[65] Erinjeri JP, Thomas CT, Samoilia A, Fleisher M, Gonen M, Sofocleous CT, Thornton RH, Siegelbaum RH, Covey AM, Brody LA, Alago W Jr, Maybody M, Brown KT, Getrajdman GI, Solomon SB. Image-guided thermal ablation of tumors increases the plasma level of interleukin-6 and interleukin-10. 2013. Journal of Vascular and Interventional Radiology. 2013;**24**(8):1105-1112. DOI: 10.1016/j.jvir.2013.02.015

[66] Ahmad F, Gravante G, Bhardwaj N, et al. Changes in interleukin-1β and 6 after hepatic microwave tissue ablation compared with radiofrequency, cryotherapy and surgical resections. American Journal of Surgery. 2010;**200**(4):500-506

[67] Schwacha MG, Schneider CP, Chaudry IH. Differential expression and tissue compartmentalization of the inflammatory response following thermal injury. Cytokine. 2002; **17**(5):266-274. DOI: https://doi.org/10.1006/cyto.2001.1003

[68] Trikha M. Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: A review of the rationale and clinical evidence. Clinical Cancer Research. 2003;**9**(13):4653-4665

[69] Hokey DA, Yan J, Hirao LA, Dai A, Boyer JD, Jure-Kunkel MN, Weiner DB. CLTA-4 blockade in vivo promotes the generation of short-lived effector CD8 T cells and a more persistent central memory CD4 T cell response. Journal of Medical Primatology. 2008;**37**(8):62-68. DOI: 10.1111/j.1600-0684.2008.00324.x

[70] Spranger S, Koblish HK, Horton B, Scherle PA, Newton R, Gajewski TF. Mechanism of tumor rejection with doublets of CTLA-4, PD-1/PD-L1, or IDO blockade involves restored IL-2 production and proliferation of CD8(+) T cells directly within the tumor microenvironment. Journal for ImmunoTherapy of Cancer. 2014;**2**(1):3. DOI: 10.1186/2051-1426-2-3

[71] Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, O7 H, Robert C, Ascierto PA, Richards JM, Lebbé C, Ferraresi V, Smylie M, Weber JS, Maio M, Konto C, Hoos A, de Pril V, Gurunath RK, de Schaetzen G, Suciu S, Testori A. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): A randomised, double-blind, phase 3 trial. The Lancet Oncology. 2015;**16**(5):522-530. DOI: 10.1016/S1470-2045(15)70122-1

[72] Waitz R, Solomon SB, Petre EN, Trumble AE, Fasso M, Norton L, Allison JP. Potent induction of tumor immunity by combining tumor Cryoablation with anti–CTLA-4 therapy. Cancer Research. 2012;**72**(2):430-439. DOI: 10.1158/0008-5472.CAN-11-1782

[73] Sabel MS, Su G, Griffith KA, Chang AE. Rate of freeze alters the immunologic response after cryoablation of breast cancer. Annals of Surgical Oncology. 2010;**17**(4):1187-1193. DOI: 10.1245/s10434-009-0846-1

[74] Yamashita T, Hayakawa K, Hosokawa M, Kodama T, Inoue N, Tomita K, et al. Enhanced tumor metastases in rats following cryosurgery of primary tumor. Gann. 1982;**73**:222-228

[75] Shibata T, Yamashita T, Suzuki K, Takeichi N, Micallef M, Hosokawa M, et al. Enhancement of experimental pulmonary metastasis and inhibition of subcutaneously transplanted tumor growth following cryosurgery. Anticancer Research. 1998;**18**(4):4443-4448

[76] Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: Clearance of apoptotic cells regulates immune responses. Nature Reviews. Immunology. 2002;**2**(12):965-975

[77] Stuart LM, Lucas M, Simpson C, Lamb J, Savill J, Lacy-Hulbert A. Inhibitory effects of apoptotic cell ingestion upon endotoxin-driven myeloid dendritic cell maturation. Journal of Immunology. 2002;**168**(4):1627-1635

[78] Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. The Journal of Clinical Investigation. 1998;**101**(4):890-898. DOI: 10.1172/JCI1112

[79] Urano M, Tanaka C, Sugiyama Y, Miya K, Saji S. Antitumor effects of residual tumor after cryoablation: The combined effect of residual tumor and a protein-bound polysaccharide on multiple liver metastases in a murine model. Cryobiology. 2003;**46**(3): 238-245

[80] Poggi G, Tosoratti N, Montagna B, Picchi C. Microwave ablation of hepatocellular carcinoma. World Journal of Hepatology. 2015;**7**(25):2578-2589. DOI: 10.4254/wjh.v7.i25.2578

[81] Ohmoto K, Yoshioka N, Tomiyama Y, Shibata N, Kawase T, Yoshida K, Kuboki M, Yamamoto S. Comparison of therapeutic effects between radiofrequency ablation and percutaneous microwave coagulation therapy for small hepatocellular carcinomas. Journal of Gastroenterology and Hepatology. 2009;**24**(2):223-227. DOI: 10.1111/j.1440-1746.2008.05596.x

[82] Poggi G, Montagna B, DI Cesare P, Riva G, Bernardo G, Mazzucco M, Riccardi A. Microwave ablation of hepatocellular carcinoma using a new percutaneous device: Preliminary results. Anticancer Research. 2013;**33**(3):1221-1227

[83] Ziemlewicz T, Hinshaw JL, Lubner MG, Brace CL, Alexander ML, Agarwal P, Lee FT. Percutaneous microwave ablation of hepatocellular carcinoma with a gas-cooled system: initial clinical results with 107 tumors. Journal of Vascular and Interventional Radiology. 2015;**26**(1):62-68. DOI: 10.1016/j.jvir.2014.09.012

[84] Ierardi AM, Mangano A, Floridi C, Dionigi G, Biondi A, Duka E, Lucchina N, Lianos GD, Carrafiello G. A new system of microwave ablation at 2450 MHz: Preliminary experience. Updates in Surgery. 2015;**67**(1):39-45. DOI: 10.1007/s13304-015-0288-1

[85] Martin RC, Scoggins CR, McMasters KM. Safety and efficacy of microwave ablation of hepatic tumors: A prospective review of a 5-year experience. Annals of Surgical Oncology. 2010;**17**(1):171-178. DOI: 10.1245/s10434-009-0686-z

[86] Lubner MG, Brace CL, Hinshaw JL, Lee FT Jr. Microwave tumor ablation: Mechanism of action, clinical results, and devices. Journal of Vascular and Interventional Radiology. 2010;**21**(8):192-203. DOI: 10.1016/j.jvir.2010.04.007

[87] Trembly BS, Douple EB, Ryan TP, Hoopes PJ. Effect of phase modulation on the temperature distribution of a microwave hyperthermia antenna array in vivo. International Journal of Hyperthermia. 1994;**10**(5):691-705. DOI: http://dx.doi.org/10.3109/02656739409022448

[88] Yu NC, Raman SS, Kim YJ, Lassman C, Chang X, Lu DS. Microwave liver ablation: Influence of hepatic vein size on heat-sink effect in a porcine model. Journal of Vascular and Interventional Radiology. 2008;**19**(7):1087-1092. DOI: https://doi.org/10.1016/j.jvir.2008.03.023

[89] Brace CL. Microwave ablation technology: What every user should know. Current Problems in Diagnostic Radiology. 2009;**38**(2):61-67. DOI: https://doi.org/10.1067/j.cpradiol.2007.08.011

[90] Li X, Liang P. Immunotherapy for hepatocellular carcinoma following thermal ablation. Journal of BUON. 2014;**19**(4):867-871

[91] Zhang H, Hou X, Cai H, Zhuang X. Effects of microwave ablation on T-cell subsets and cytokines of patients with hepatocellular carcinoma. Minimally Invasive Therapy & Allied Technologies. 2017;**26**(4):207-211. DOI: 10.1080/13645706.2017.1286356

[92] Li L, Wang W, Pan H, Ma G, Shi X, Xie H, Liu X, Ding Q, Zhou W, Wang S. Microwave ablation combined with OK-432 induces Th1-type response and specific antitumor immunity in a murine model of breast cancer. Journal of Translational Medicine. 2017;**15**(1):23. DOI: 10.1186/s12967-017-1124-9

[93] MA Y, Liang P, XL Y, Han ZY, Dong XJ, Wang YU, Chenq C, Li X. Multiple courses of immunotherapy with different immune cell types for patients with hepatocellular carcinoma after microwave ablation. Experimental and Therapeutic Medicine. 2015; **10**(4):1460-1466. DOI: 10.3892/etm.2015.2681

[94] Chen Z, Shen S, Peng B, Tao J. Intratumoural GM-CSF microspheres and CTLA-4 blockade enhance the antitumour immunity induced by thermal ablation in a subcutaneous murine hepatoma model. International Journal of Hyperthermia. 2009;**25**(5):374-382. DOI: 10.1080/02656730902976807

[95] Ryoma Y, Moriya Y, Okamoto M, Kanaya I, Saito M, Sato M. Biological effect of OK-432 (picibanil) and possible application to dendritic cell therapy. Anticancer Research. 2004; **25**(5):3295-3301

[96] Zhou P, Liang P, Dong B, Yu X, Han Z, Xu Y. Phase I clinical study of combination therapy with microwave ablation and cellular immunotherapy in hepatocellular carcinoma. Cancer Biology & Therapy. 2011;**11**(5):450-456