High intensity focused ultrasound ablation and antitumor immune response

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The ideal cancer therapy not only induces the death of all localized tumor cells without damage to surrounding normal tissue, but also activates a systemic antitumor immunity. High intensity focused ultrasound (HIFU) has the potential to be such a treatment, as it can non-invasively ablate a targeted tumor below the skin surface, and may subsequently augment host antitumor immunity. This paper is to review increasing pre-clinical and clinical evidence linking antitumor immune response to HIFU ablation, and to discuss the potential mechanisms involved in HIFU-enhanced host antitumor immunity. The seminal studies performed so far indicate that although it is not possible to conclude definitively on the connection between HIFU treatment and antitumor immune response, it is nonetheless important to conduct extensive studies on the subject in order to elucidate the processes involved. © *2013 Acoustical Society of America*. [http://dx.doi.org/10.1121/1.4812893]

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I. INTRODUCTION

As an alternative approach to surgical intervention, the thermal ablation of tumors with high intensity focused ultrasound (HIFU) has received increasingly widespread interest in the local treatment of solid malignancy. It employs acoustic energy to raise the temperature between 56 °C and 100 °C for *in situ* destruction of a targeted tumor, instead of local tumor removal. In addition, non-thermal effects such as cavitation can induce the local destruction of the tissue due to microbubble-induced high pressures and temperatures. The main advantage of HIFU is that it is less invasive than a surgical procedure, resulting in an associated reduction in mortality, morbidity, hospital stay, cost, and improved quality of life for cancer patients. As curative and palliative treatments, HIFU has been increasingly used in clinical practice to treat patients with solid tumors, including those of the prostate, liver, kidney, breast, pancreas, bone, and soft tissues.

It has been noted that large amounts of tumor debris remain *in situ* after HIFU ablation. As a normal process of healing response, the tumor debris is gradually reabsorbed by the individual patient, which takes a period ranging from months to a few years. It is still unclear what kind of biological significance may exist during the absorption of the ablated tumor. However, some studies have shown that an active immune response to the heat-treated tumor could be developed after thermal ablation, and the host immune system could become more responsive to the tumor cells.[1] This may lead to a potential procedure that reduces or perhaps eliminates metastases, and prevents local recurrence in cancer patients who have had original dysfunction of antitumor immunity before treatment. In this paper we review the pre-clinical and clinical studies that focused on the host immune responses after HIFU ablation of a tumor, and analyze experimental and clinical data available to assess whether there would be the potential for understanding this complex phenomenon.

II. BIOLOGICAL EFFECTS OF HIFU

A. Direct thermal and non-thermal effects

The effects of thermal ablation on a targeted tumor are determined by increased temperatures due to thermal energy deposition, rate of removal of heat, and the specific thermal sensitivity of the tissue. As the tissue temperature rises, the time required to achieve irreversible cellular damage decreases exponentially. At temperatures between 50 °C and 55 °C, cellular death occurs instantaneously in cell culture.[2] Protein denaturation, membrane rupture, cell shrinkage, pyknosis, and hyperchromasia occur *ex vivo* between 60 °C and 100 °C, leading to almost immediate coagulation necrosis.[3] Tissue vaporization and boiling are superimposed on this process at temperatures higher than 105 °C. Carbonization, charring, and smoke generation occur while the temperature is over 300 °C.[4] In addition, acoustic cavitation, one of the mechanical effects induced by HIFU ablation, is the most important non-thermal mechanism for tissue disruption.[5] Small gaseous nuclei existing in subcellular organelles and fluid in tissue are the sources of cavitation, which can expand and contract under the influence of the acoustic pressure. During the collapse of bubbles, the acoustic pressure, shear stress, and subsequently high temperature can induce the local destruction of a targeted tissue.[6]

B. Thermal effects on tumor blood vessels

Structural and functional changes are directly observed in tumor blood vessels after thermal ablation. These changes are not as well described as thermal effects on the tissues, but they rely on varying temperatures. At temperatures between 40 °C and 42 °C, there is no significant change in

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tumor blood flow after 30 to 60 min exposure.[7] Beyond 42 °C to 44 °C, there is an irreversible decrease in tumor blood flow, with vascular stasis and thrombosis, resulting in heat trapping and progressive tissue damage.[8] When temperatures exceed 60 °C, immediate destruction of tumor microvasculature occurs.[9] It cuts the blood supply to the tumor directly through the cauterization of the tumor feeder vessels, leading to deprivation of nutrition and oxygen. Thus, tissue destruction can be enhanced by the damage caused by thermal ablation to tumor blood vessels.

C. Indirect effects on the tumor cells

Indirect injury is a secondary damage to the tissue, which progresses after the cessation of thermal ablation stimulus.[10] It is based on histological evaluation of tissue damage at various time points after thermal ablation. The full extent of the secondary tissue damage becomes evident 1 to 7 days after thermal ablation, depending on the model and energy source used.[11,12] The exact mechanism of this process is still unknown. However, it may represent a balance of several promoting and inhibiting mechanisms, including induction of apoptosis, Kupffer cell activation, and cytokine release.

Cellular apoptosis may contribute to the progressive injury of tissue after thermal ablation. It is well-established that apoptosis increases in a temperature-dependent manner, and temperatures between 40 °C and 45 °C cause inactivation of vital enzymes, thus initiating apoptosis of tumor cells.[13,14] Most thermal ablation techniques create a temperature gradient that progressively decreases away from the site of probe insertion. The induction of apoptosis at a distance from the heat source may potentially contribute to the progression of injury. An increased rate of apoptosis is observed in the liver 24 h after HIFU ablation. The stimulation of apoptosis may be directly induced by temperature elevations, alterations in tissue microenvironment, and the release of various cytokines after thermal ablation.

Kupffer cell activity may be one of the major factors involved in the progressive injury after thermal ablation. Heat triggers Kupffer cells to secret interleukin-1 (IL-1)[15] and tumor necrosis factor-α (TNF-α),[16] which are known to have *in vivo* antitumor activity[17] and to increase apoptosis in cancer cells.[14] Kupffer cells also induce the production of interferon that augments the liver-associated natural killer (NK) cell activity.[18]

Thermal ablation may induce both regional and systemic production of cytokines through activation of inflammatory cells. Compared with controls, the circulating level of interferon-γ (IFN-γ) and vascular endothelial growth factor levels markedly increase after radio frequency (RF) ablation.[19,20] The increased level of IL-1 and TNF-α is also observed after RF ablation.[21] These cytokines may have direct cytotoxic effects such as inducing tumor endothelial injury and rendering tumor cells more sensitive to heat-induced damage.[22,23] However, contrasting results are obtained for TNF-α level in two studies[20,24] and IL-1 level in one study,[25] which remains unchanged after thermal ablation.

III. HIFU-INDUCED ANTITUMOR IMMUNE RESPONSE

A. Experimental evidence

As shown in Table I, there is increasing evidence from animal studies that indicate that HIFU may modulate host antitumor immunity after tumor ablation. Yang and colleagues[26] used HIFU to treat C1300 neuroblastoma implanted in mouse flanks, followed by the re-challenge of the same tumor cells. A significantly slower growth of re-implanted tumors was observed in these mice while compared with the controls. After HIFU treatment, the cytotoxicity of cytotoxic T lymphocytes (CTLs) and the number of activated tumor-specific CTLs was significantly increased in the H22 tumor bearing mice treated with HIFU. An adoptive transfer of the activated-lymphocytes could provide better long-term survival and lower metastatic rates in the mice re-challenged by the same tumor cells while compared with sham-HIFU and control groups, indicative of that HIFU ablation could activate tumor-specific T lymphocytes, thus inducing antitumor cellular immunity in the mice.[27] Similar results were confirmed in the mice implanted MC-38 colon adenocarcinoma and melanoma after HIFU ablation. HIFU treatment could also induce an enhanced CTL activity *in vivo*, thus providing protection against subsequent tumor re-challenge.[28,29] In addition, HIFU could enhance the infiltration of dendritic cells (DCs) in the treated tumor and subsequent migration to the draining lymph nodes. Compared to thermal HIFU treatment, antitumor immunity induced by mechanical HIFU treatment, which was a pulsed HIFU exposure with no significantly elevated temperature increase in tumor tissue that will lead to thermal necrosis, was significantly stronger in terms of the activation of DCs and CTLs, as well as superior protection against tumor re-challenge.[29]

After HIFU ablation, large amounts of tumor debris remain *in situ*, and the host gradually reabsorbs them as the normal process of a healing response. Using a murine hepatocelluar carcinoma model, Zhang and colleagues[30] demonstrated that the remaining tumor debris induced by HIFU could be immunogenic as an effective vaccine to elicit tumor-specific immune responses, including induction of CTL cytotoxic activity, enhanced activation of DCs, and protection against a lethal tumor challenge in naive mice. When the tumor debris was loaded with immature DCs, it could significantly induce maturation of DCs, and increased cytotoxicity and TNF-α and IFN-γ secretion by CTL, thus initiating a host specific immune response after H22 challenge in the vaccinated mice.[31] Immediately after HIFU exposure to MC-38 colon adenocarcinoma cells *in vitro*, the release of endogenous danger signals including HSP60 (HSP = heat shock protein) was observed from the damaged cells. These signals could subsequently activate antigen presenting cells (APCs), leading to an increased expression of co-stimulatory molecules and enhanced secretion of IL-12 by the DCs, and an elevated secretion of TNF-α by the macrophages.[32] In addition, HIFU could up-regulate an *in vitro* and *ex vitro* molecule expression of HSP70,[33,34] which are intracellular molecular chaperones that can enhance tumor cell immunogenicity, resulting in potent cellular immune responses.

TABLE I. Antitumor immune response to HIFU alone in animal studies.

Author	Year	Tumor cell lines and model	HIFU parameters	Endpoint	Results	Additional observation
Yang *et al.* (Ref. 26)	1992	C1300 neuroblastoma in Ajax (A/J) mice	Frequency: 4 MHz; acoustic intensity: 550 W/cm^2	Resistance to re-challenge	Significant inhibition of tumor growth in mice treated with curative HIFU compared to the untreated control	Single and repeated HIFU could prolong survival rates in the tumor bearing mice
Xia *et al.* (Ref. 27)	2012	H22 hepatocellular carcinoma in C57BL/6 J mice	Frequency: 9.5 MHz; acoustic power: 5 W; exposure time: 180 to 240 s	Measurement of CTLs cytotoxicity, and resistance to re-challenge after adoptive transfer of the activated-lymphocytes	A significant increase of CTLs cytotoxicity, and superior protection after adoptive immunotherapy	Significantly increased number of activated tumor-specific CTLs
Xing *et al.* (Ref. 28)	2008	B16-F10-Luc-G5 melanoma in C57BL/6 mice	Frequency: 3.3 MHz	Measurement of CTLs cytotoxicity	A significant increase of CTLs cytotoxicity in HIFU-treated mice compared to the untreated control.	HIFU could not increase the risk of distant metastasis
Hu *et al.* (Ref. 29)	2007	MC-38 colon adenocarcinoma in C57BL/6 mice	Frequency: 3.3 MHz	Resistance to re-challenge, and cytotoxicity assays of splenic lymphocytes	Superior protection and tumor-specific lymphocyte mediated cytotoxicity after both thermal and mechanical HIFU treatments. Antitumor immunity induced by cavitation-based HIFU was stronger compared to thermal HIFU	HIFU could enhance DCs infiltration in the treated tumor and subsequent migration to draining lymph nodes
Zhang *et al.* (Ref. 30)	2010	H22 hepatocellular carcinoma in C57BL/6 J mice	Frequency: 9.5 MHz; acoustic power: 5 W; exposure time: 180 to 240 s	Resistance to re-challenge and measurement of DCs activation and CTLs cytotoxicity after immunization of HIFU-generated tumor vaccine	A significant increase of CTLs cytotoxicity and DCs activation, and superior protection in mice immunized with HIFU-generated tumor vaccine while compared with the controls	HIFU treatment alone could enhance CTLs cytotoxicity and resistance to re-challenge
Deng *et al.* (Ref. 31)	2010	H22 hepatocellular carcinoma in C57BL/6 J mice	Frequency: 9.5 MHz; acoustic power: 5 W; exposure time: 180 to 240 s	Measurement of DCs activation, CTLs cytotoxicity and resistance to re-challenge after immunization of DCs loaded with HIFU-treated tumor	A significant increase of DCs activation and CTLs cytotoxicity with inhibition of tumor growth in mice immunized by DCs loaded with HIFU-treated tumor while compared with the controls	
Hu *et al.* (Ref. 32)	2005	MC-38 mouse colon adenocarcinoma cells *in vitro*	Frequency: 1.1 MHz; acoustic pressure: P^+ 12.0/P^- 6.7 MPa or P^+ 31.7 /P^- 10.7 MPa; duty cycle: 30% or 3%; exposure time: 5 or 30 s	Measurement of endogenous danger signals released from HIFU-treated cells, and subsequent activation of APCs	Release of ATP and HSP60 from HIFU-treated tumor cells, with subsequent activation of DCs and macrophages	
Kruse *et al.* (Ref. 33)	2008	Transgenic reporter mouse for Hsp70-luc2AeGFP	Frequency: 1.5 MHz; acoustic intensity: 53 to 352 W/cm^2	Gene expression of skin HSP70 after 1-s HIFU treatment	Up-regulated expression of HSP70 after HIFU treatment	
Hundt *et al.* (Ref. 34)	2007	Transfected Hsp70-luc M21 melanoma, NIH3T3 mouse fibroma and SCCVII mouse squamous cell carcinoma cells *in vitro*	Frequency: 1 MHz; acoustic intensity: 28 to 179 W/cm^2; total energy: 8404 to 53 834 W/cm^2	Gene expression of HSP70 in tumor cells after either thermal stress or HIFU treatment	Increased expression of HSP70 after both thermal stress and HIFU treatment, but a higher expression observed at HIFU-induced lower temperature than thermal stress alone	

TABLE I. (*Continued.*)

Author	Year	Tumor cell lines and model	HIFU parameters	Endpoint	Results	Additional observation
Liu *et al.* (Ref. 35)	2010	B16 melanoma in C57BL/6 mice	Frequency: 3.3 MHz; acoustic pressure: P^+ 19.5/P^- 7.2 MPa; exposure time: 4 s	Measurement of DCs infiltration and maturation in HIFU-treated tumor	A significant increase of local DCs infiltration and maturation after HIFU treatment compared to the controls	Sparse-scan HIFU was more effective than dense-scan HIFU in enhancing DCs infiltration and maturation *in situ*
Zhou *et al.* (Ref. 36)	2007	H22 hepatocellular carcinoma in Chinese Kun Ming mice	Frequency: 9.5 MHz Acoustic power: 5 W Exposure time: 180-240 s	Resistance to re-challenge after immunization of HIFU-treated tumor vaccine	Significant protection in mice immunized with HIFU-treated tumor compared to heat-treated tumor group	A significant increase of $CD4^+$ levels and $CD4^+/CD8^+$ ratio in both HIFU and thermal groups

The potency of DC infiltration and activation from mechanical lysis and a sparse-scan HIFU was much stronger than that from thermal necrosis and a dense-scan HIFU exposure, suggesting that optimization of HIFU ablation strategy may help to enhance immune response after treatment.[35] Heat and acoustic cavitation are two major mechanisms involved in HIFU-induced tissue damage, and cavitation is a unique effect of HIFU while compared with other thermal ablation techniques. It causes membranous organelles to collapse, including mitochondria and endoplasmic reticulum, cell and nuclear membrane. This breaks up the tumor cells into small pieces, on which the tumor antigens may remain intact, or lead to the exposure of an immunogenic moiety that is normally hidden in tumor antigens.[1] Zhou and colleagues[36] used either heat-exposed or HIFU-treated H22 tumor vaccine to inoculate naive mice. The vaccination times were 4 sessions, once a week for 4 consecutive weeks, and each mouse was challenged with H22 tumor cells 1 week after the last vaccination. They found that the HIFU-treated tumor vaccine could significantly inhibit tumor growth, and increase survival rates in the vaccinated mice, suggesting that acoustic cavitation could play an important role to stimulate the host antitumor immune system.

B. Clinical evidence

Emerging clinical results revealed that systemic cellular immune response was observed in cancer patients after HIFU treatment, as shown in Table II. Rosberger and colleagues[37] reported five consecutive cases of posterior choroidal melanoma treated with HIFU. Three patients had abnormal and two patients normal $CD4^+/CD8^+$ (CD = cluster of differentiation) ratios before treatment. One week after treatment, the ratio in two patients reverted to normal, while another was noted to have a 37% increase in his $CD4^+$ T-cells relative to his $CD8^+$ cells. Wang and Sun[38] used multiple-session HIFUs to treat 15 patients with late-stage pancreatic cancer. Although there was an increase in the average values of the NK cell and T lymphocyte and subset in 10 patients after HIFU treatment, a significant statistical difference was observed in only NK cell activity before and after HIFU treatment ($p < 0.05$). Wu and colleagues[39] observed changes in circulating NK, T lymphocyte, and subsets in 16 patients with solid malignancy before and after HIFU treatment. The results showed a significant increase in the population of $CD4^+$ T lymphocytes ($p < 0.01$) and the ratio of $CD4^+/CD8^+$ ($p < 0.05$) after HIFU treatment. The abnormal levels of $CD3^+$ lymphocytes returned to normal in two patients, $CD4^+/CD8^+$ ratio in three, $CD19^+$ lymphocytes in one, and NK cell in one, respectively, compared to the values in the control group. In addition, serum levels of immunosuppressive cytokines including vascular endothelial growth factor (VEGF), TGF-β1 (TGF = transforming growth factor) and TGF-β2 were significantly decreased in the peripheral blood of cancer patients after HIFU treatment, indicating that HIFU may lessen tumor-induced immunosuppression, and renew host antitumor immunity.[40]

Clinical evidences suggest that HIFU treatment may also enhance local antitumor immunity in cancer patients.

TABLE II. Antitumor immune response to HIFU alone in clinical studies.

Author	Year	Tumor	HIFU parameters	Endpoint	Results	Additional observation
Rosberger *et al.* (Ref. 37)	1994	5 patients with choroidal melanoma	Frequency: 4.6 MHz; acoustic intensity: 2 W/cm^2	Measurement of T lymphocyte and subsets in peripheral blood	CD4$^+$/CD8$^+$ ratio reverted to normal after HIFU in 2 of 3 patients with previously abnormal CD4$^+$/CD8$^+$ ratio	A 37% increase in CD4$^+$ T-cells relative to CD8$^+$ cells in the remaining one patient
Wang and Sun (Ref. 38)	2002	15 patients with pancreatic cancer	Acoustic power: 0.5 to 1.6 KW; each exposure time: 30 to 80 s	Measurement of T lymphocyte and subsets, and NK cell activity in peripheral blood	A significant increase of NK cell activity after HIFU treatment	An increase of CD3$^+$, CD4$^+$ and CD4$^+$/CD8$^+$ ratio in 10 patients after HIFU treatment, but no statistical significance
Wu *et al.* (Ref. 39)	2004	16 cancer patients including 6 with osteosarcoma, 5 with hepatocellular carcinoma and 5 with renal cell carcinoma	Frequency: 0.8 MHz; acoustic intensity: 5000-20 000 W/cm^2	Measurement of circulating NK, T lymphocyte and subsets	A significant increase of CD4$^+$ T cells and CD4$^+$/CD8$^+$ ratio after HIFU treatment	The abnormal levels of CD3$^+$ returned to normal in 2 patients, CD4$^+$/CD8$^+$ ratio in 3, CD19$^+$ cell in 1, and NK cell in 1
Zhou *et al.* (Ref. 40)	2008	15 cancer patients including 13 with liver cancer and 2 with sarcoma	Frequency: 0.8 MHz; acoustic intensity: 5000 to 20 000 W/cm^2	Measurement of serum immunosuppressive cytokines in peripheral blood	A significant decrease of serum VEGF, TGF-β1 and TGF-β2 levels after HIFU treatment	
Madersbacher *et al.* (Ref. 41)	1998	5 patients with prostate cancer and 4 patients with bladder cancer	Frequency: 4 MHz; acoustic intensity: 1260 to 2200 W/cm^2	Measurement of HSP27 expression in HIFU-treated tumor and prostate tissue	A significant increase of HSP27 expression after HIFU treatment compared to the controls	
Kramer *et al.* (Ref. 42)	2004	6 patients with prostate cancer	Frequency: 4 MHz; acoustic intensity: 1260 to 2200 W/cm^2	Measurement of expression of HSPs, and Th1- and Th2-cytokine release of TILs in HIFU-treated tumor	A significantly up-regulated expression of HSP72, HSP73, and glucose GRP75 and 78, with a significant increase of TILs-released IL-2, IFN-γ, and TNF-α after HIFU treatment	A significant decrease of TILs-released Th2-cytokine (IL-4, -5, -10) after HIFU treatment
Wu *et al.* (Ref. 43)	2007	23 patients with breast cancer	Frequency: 4 MHz; acoustic intensity: 1260 to 2200 W/cm^2	Measurement of expression of 13 proteins on tumor cells including HSPs	A 100% positive rate of HSP70 in HIFU-treated cancer cells compared to the control	Various expressions of ER, PR, CA15-3, VEGF, TGF-β1, TGF-β2, IL-6, IL-10 and EMA in HIFU-treated tumor, with no expression of PCNA, MMP-9 and CD44v6
Xu *et al.* (Ref. 44)	2009	23 patients with breast cancer	Frequency: 4 MHz; acoustic intensity: 1260 to 2200 W/cm^2	Measurement of APCs infiltration and activation in HIFU-treated tumor	A significant increase of local infiltration and activation of DCs and macrophages compared to the control	
Lu *et al.* (Ref. 45)	2010	23 patients with breast cancer	Frequency: 4 MHz; acoustic intensity: 1260 to 2200 W/cm^2	Measurement of TILs infiltration and activation in HIFU-treated tumor	A significant increase of tumor-infiltrating CD3, CD4$^+$, CD8$^+$ T cells, CD4$^+$/CD8$^+$, B lymphocytes, NK cells, FasL+, granzyme+, and perforin+ TILs while compared with the control	

Kramer and colleagues[41,42] found that HIFU treatment could alter the presentation of tumor antigens in prostate cancer patients, which was most likely to be stimulatory. A histological examination showed a significantly up-regulated expression of HSP72, HSP73, and glucose regulated protein (GRP) 75 and 78 at the border zone of HIFU treatment in prostate cancer. Heated prostate cancer cells exhibited increased Th1-cytokine (IL-2, IFN-γ, TNF-α) release but decreased Th2-cytokine (IL-4, -5, -10) release of tumor infiltrating lymphocytes (TILs). The up-regulated expression of HSP70 was confirmed in the tumor debris of breast cancer after HIFU ablation,[43] indicating that HIFU may modify tumor antigenicity to produce a host immune response.

Xu and colleagues[44] found the number of tumor-infiltrating APCs including DCs and macrophages increased significantly along the margin of HIFU-treated human breast cancer, with an increased expression of human leukocyte antigen-DR, CD80, and CD86 molecules. Activated APCs may take up the HSP-tumor peptide complex, which remains in the tumor debris, and present the chaperoned peptides directly to tumor-specific T lymphocytes with high efficiency, resulting in potent cellular immune responses against tumor cells after HIFU treatment. Furthermore, HIFU could induce significant infiltration of TILs in human breast cancer, including CD3, $CD4^+$, $CD8^+$, B lymphocytes, and NK cells. The number of activated CTLs expressing Fas ligand ($FasL^+$), $granzyme^+$, and $perforin^+$ significantly increased in the HIFU-treated tumor, suggesting that specific cellular antitumor immunity could be locally triggered after HIFU treatment.[45]

IV. DISCUSSION AND CONCLUSION

As a non-invasive therapy, HIFU has been increasingly used in clinical practice for the local treatment of solid malignancy. Beyond optimization of technical and physiological parameters, it is clear that HIFU ablation should be undertaken when there is precise knowledge not only of the number and location of the lesions, but also of the biological characteristics and natural history of the tumor. The goal of tumor therapy is that all cancer cells should be completely killed in the patient's body. A similar multidisciplinary approach including other modalities is important in the treatment of solid malignancies. For patients with cancer, the therapeutic strategy for the disease should be a multiple treatment plan, which includes local treatments such as surgery and radiotherapy, and systemic therapy such as chemotherapy and immunotherapy. So, the success achieved in the application of HIFU treatment is mainly dependent not only on the HIFU technique, but also on better understanding of the natural characteristics of tumors.

Recent studies support academic evidence that HIFU ablation may elicit a systemic antitumor immune response. They range from anecdotal observations in a clinical setting to a variety of animal models and correlative immune studies in patients undergoing HIFU treatment. It is not surprising that there is great concern about a close relationship between HIFU ablation and antitumor immune response as thermal ablation may have the potential to be both local and systemic therapies. It may lead to a post-ablative procedure that reduces or perhaps eliminates distant disease, and prevents local recurrence through the immune system in cancer patients who have had original dysfunction of antitumor immunity after ablation.

Although the mechanism for HIFU-induced antitumor immune response is still unclear, several possibilities have been hypothesized based on previous results. First, host immune suppression induced by tumor cells may be lessened or relieved after thermal ablation as the tumor is completely ablated, leading to renewed host antitumor immunity. Second, thermal ablation may modify tumor antigenicity, and up-regulate the expression of HSPs, which act as tumor vaccines to produce potent cellular immune responses. Third, cytokines are secreted by immune cells at the inflammatory margin of the ablation-treated region, presenting a milieu for the development of mature CTLs. Finally, large amounts of cellular debris are gradually phagocytized by macrophages and other cells that can function as APCs. However, as microwave, laser, and RF can also induce antitumor immune response, further studies are needed to investigate the differences between HIFU-induced immunity and the immune response triggered by other ablation techniques. In addition, cryotherapy uses extreme cold to freeze a targeted tumor in the form of an "ice-ball," which causes rupture of cell membranes, protein denaturation, and cellular death. This process is almost similar to cavitation involved in HIFU that cancer cells are broken up into small pieces due to the destruction of cell membranes. Therefore, it is necessary to compare mechanical HIFU with cryotherapy in the induction of antitumor immune response in the future.

It is increasingly apparent that HIFU ablation alone may not be sufficient to generate a clinically relevant immune response and to stimulate the host immune system consistently. A strategy to combine HIFU ablation with active immunological stimulation such as immunoadjuvants may augment the efficacy of HIFU-induced antitumor immunity specifically against the targeted tumors, if the destruction of tumors releases tumor antigens or improves tumor immunogenicity. This combined approach may become an important part in the HIFU treatment of solid malignancy.

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