Noninvasive Treatment of Breast Cancer Using High-Intensity Focused Ultrasound

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Breast conserving surgery is a standard procedure for breast cancer since 1970. Now noninvasive therapy with few complications is preferred. High-intensity focused ultrasound (HIFU) has been developed to ablate solid tumor or cancer noninvasively. Its clinical outcome on breast cancer in China and Europe as well as the induced vascular destruction and immune response are summarized. In summary, HIFU is a promising therapeutic modality for breast cancer.

Keywords: High-Intensity Focused Ultrasound, Breast Cancer, Coagulative Necrosis, Vessel Destruction, Immune Response.

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1. BREAST CANCER AND CONVENTIONAL TREATMENT

Breast cancer is one of the most popular female malignant diseases, 411,000 annual deaths accounting for 14% of all female cancer deaths world-wide, and is the fifth cancer mortality over-all. There were 1.15 million new cases in 2002, mostly in the developed countries (361,000 in Europe and 230,000 in North American) with the average survival rate of 73% and 57% in developed and developing countries, respectively, which may be due to the routine screening programs in the West.1

Radical mastectomy, breast amputation with/without the pectoral muscle removal, and requirement of skin graft, was introduced by William Halsted in 1894 based on his hypothesis that local therapy of breast cancer, chest wall, and regional lymph nodes is beneficial for survival,2 and has been the mainstream for about 80 years.3,4 The failure in a satisfactory surgical margin usually leads to a high local recurrence ratio. In the 1970s, a further understanding of breast cancer introduced a local excision.5 Since then, breast-conserving surgery followed by radiotherapy has become gold standard for patients with localized early-stage breast cancer,6 and it can also be in conjunction with interstitial laser coagulation, radiofrequency, cryotherapy, interstitial radiotherapy, chemotherapy, and hormonal therapy.7 Besides the advance in therapy, nationwide breast cancer screening programs became routine and popular in many developed countries since the 1990s so that the diagnostic percentage of small carcinomas has increased. Although at a low morbidity rate, breast-conserving surgery also introduces certain complications, such as bleeding and infections.8 In addition, because of the surgical resection requires a 1 cm margin of normal tissue and use of postoperative radiation women with cosmetic worries consider this modality suboptimal. It is noted that both the mastectomy and breast conservation surgery have similar long-term survival rate.5,8

In the past decade, imaging-guided minimally and noninvasive therapies are being developed. Compared with surgical methods, they have several potential advantages of reduced recovery time and hospital stay, fewer complications (i.e., infections, bleeding, scar formation), less anesthesia or even under conscious sedation in outpatient setting, and possibly also less healthcare cost.9,10 Two prerequisites should be met before their acceptance by the physician. First, the imaging guidance must display exact anatomy of the region-of-interest (i.e., tumor and surrounding normal tissue). Second, delivery and deposition of energy must be precise for the lesion generation only without damage to the vital tissue (i.e., vessel and nerve) and intervening part.

2. HIFU PRINCIPLE, SYSTEM, AND PROCEDURE

The use of an extracorporeal focused ultrasound energy in medical therapy began in 1942.11 The acoustic intensity of high-intensity focused ultrasound (HIFU) at the focus is...
1,000–20,000 W/cm² with peak compressive pressure up to 70 MPa, peak rarefractional pressure up to 20 MPa, and a small focal region (i.e., ~1 mm in diameter and ~10 mm in length), which minimizes the potential for thermal damage to intervening tissue. Absorption of the acoustic energy in the local tissue causes a rapid temperature rise of more than 60 °C within seconds and leads to instantaneous and irreversible cell death via coagulative necrosis with a sharp demarcation (i.e., ~50 μm) between the treated and normal tissue (Fig. 1). After the development of medical diagnostic modality, such as ultrasound (US) and magnetic resonance imaging (MRI), for real-time targeting, guidance of HIFU energy deposition, and rapid in-situ assessment of the coagulative necrosis, digital computer and automatic control in the 1980s, practical and commercial HIFU system became possible in the middle 1990s. Breast has an excellent acoustic window for the US beam propagation and can also be easily immobilized. Compared to minimally invasive therapeutic modalities, HIFU can generate necrosis in any tumors (i.e., size and shape) in a well-controlled and highly precise manner.12–14

Breast cancer patients are usually classified using numerical subsets of the TNM components (T: the extent of the primary tumor, N: the absence or presence and extent of regional lymph node metastasis and M: the absence or presence of distant metastasis) to reveal the clinical stage of the malignant disease, which was initially developed to stage bone sarcoma.15 The patient selection criteria were: invasive breast cancer (T1−2N0−2M0) proven in histology, single palpable tumors with size no larger than 6 cm, visible lesion boundaries in color Doppler US circumscribed with distance from skin or rib cage and nipple more than 0.5 cm and 2 cm, respectively, at least 18 years old, no breast implants, stable haematogenic parameters, no extensive inaductal components (EIC), no extensive calcifications in mammography, no multicentric disease and prior local therapy, no history of active myocardial infarction in the last 6 months. Follow-up examinations on skin burns, local pain, discomfort, mammary oedema, haemorrhage or infection, and fever were used to evaluate the HIFU-induced complications.

A diagnostic US imaging probe integrated concentrically and confocally with the HIFU transducer can monitor the target by real-time sonography, guide HIFU energy deposition, and assess acute coagulative necrosis during the ablation. The shortcoming of US guidance is the under-estimation of breast tumor size,16 which makes monitoring the efficacy and safety of HIFU possible. Thus, MRI is the only FDA-approval monitoring modality for the neoadjuvant, adjuvant, or palliative HIFU ablation for patients with uterine fibroids.

All parts involved in the MRI-guided HIFU ablation (a HIFU transducer, a MRI coil, and a hydraulic positioning system) should be nonmetal materials compatible in the magnetic field. The HIFU transducer could accommodate the breast in a bowl shape. The treatment planning (power, transducer motion, and focus position) intends for an optimal beam pattern in such a manner that the multiple HIFU foci covers the entire target volume.

In the planning stage, T2w images turbo spin echo and native T1w 3D flash images with a high spatial resolution were taken to define the anatomical baseline and target volume. T2w sequences with interval of 10 min during the ablation were used to monitor the development of edema while the T1w perfusion was applied postoperatively with use of paramagnetic contrast agent gadolinium. Correlating coordinates of the MRI diagnosis and HIFU therapy was realized using MRI-visible markers attached to the patients. The T1w thermal imaging with the temperature resolution of 2–3 °C for a single sonication was applied because of its reliability in fatty tissue, especially for the elder breast cancer.

Yufeng Zhou got his MS and Ph.D. degrees from State Key Laboratory of Modern Acoustics at Nanjing University China in 1999, and Duke University USA in 2003, respectively. He served as assistant professor at Nanyang Technological University, Singapore. His research interests include medical ultrasound therapy, ultrasound-mediated drug delivery, ultrasound imaging, nondestructive evaluation/testing and other acoustic applications.
3. RESULTS

A 56-year-old menopausal female with an invasive grade 2 ductal carcinoma underwent MRI-guided HIFU ablation. The skin at the ultrasound wave entry site had no visible changes, and no local or systemic symptoms were found 3 months later with the adjuvant radiotherapy. Despite the absence of anesthesia, no pain or discomfort was experienced by the patient during or after HIFU therapy except a mild pressure sensation in the treatment region.¹⁹ The treated tumor was partially necrotic, shown as the lack of nuclear staining and mostly sublethal damage with chromatin clumping in histology, at the spatial accuracy of 1–2 mm based on the MRI coordinates (Fig. 4).

A total of 48 breast cancer patients \( (T_1 - 2N_0 - 2M_0) \) were randomized to the control group for modified radical mastectomy and the extracorporeal HIFU ablation group followed by modified radical mastectomy.²¹ Mild local pain, warmth, and sensation of pressure in the target occurred in 14 patients, but only 4 of them were given 3–5 days oral analgesics. 1 patient had slight skin burn, but recovered entirely 10 days later. No bleeding or infection of the treated breast was found. Although slight changes were found on both unenhanced T1w and T2w MR images postoperatively, the lack of contrast uptake in the treatment region (breast cancer and 1.5–2.0 cm margin) via the enhanced-MRI as indicator of coagulative necrosis (Fig. 4).²¹

22 of 106 biopsy-proven breast cancer patients received breast conservation surgery with adjuvant chemotherapy, axillary node dissection or/and radiotherapy after extracorporeal HIFU ablation.²² 5–10% patients had 5–7 days of low-grade fever up to \( 38.5^\circ \text{C} \) and some had a severe fever up to \( 39.5^\circ \text{C} \) for 2–3 weeks postoperatively, which seems to correlate with the volume of destruction.²² Minor or moderate skin burns are less than 5% with appropriate operation. 20–30% patients experienced slight and mild local pain within a week postoperatively and only 5–10%
patients needed oral analgesics for 3–5 days. No tumor bleeding or rupture of the large blood vessels was found. Absence of viable tumor was shown as no radioisotope uptake in SPECT after HIFU, and the coagulative necrosis shrunk by 20–50% in volume at 6–12 months postoperatively. Half of ablated tumor was reabsorbed within 1–2 years post-operatively. All patients were alive at the last follow-up (10–36 months) and all but one is disease-free.

22 patients (T1N0M0: 1, T2N0M0: 9, T2N1M0: 8, T2N1M1: 1, 36–68 years with average age of 48.6 years) with breast cancer (tumor size varying 2–4.8 cm with the average of 3.4 cm) were involved in a non-randomized prospective trial and underwent chemotherapy, radiotherapy, and tamoxifen postoperatively. After an average follow-up of 54.8 months, the 5-year disease-free survival was 95% with 1 death (5%) and 2 local recurrences (9%). There was no skin burns or serious bleeding or infection. A heterogeneous brightness increase in sonography and the absence of blood flow in color Doppler US in the treatment region were observed in 15 and 19 patients, respectively. Maximum tumor size in both transverse and longitudinal dimensions was assessed sonographically. During the follow-up, disappearance and size reduction of the treated tumor were found in 8 and 12 patients, respectively: 2 patients had an initial reduction but subsequent size increase because of local recurrence (Table I). Reduction in tumor size was obvious in the first 12 months, but less remarkable (stable size in some cases) between 36 and 60 months (Fig. 6).

In general, although MRI is more reliable for breast cancer delineation, the success rate of MRI-guided HIFU ablation is not high as that under the guidance of sonography, which may be due to larger ablation margin used.

Single photon emission computed tomography (SPECT) with a radioisotope tracer can assess tissue function. 5 of 6 patients had positive breast lesions in Tc-99m sestamibi SPECT, including 1 patient with 2 lesions and 1 had a negative imaging. The disappearance of contrast uptake after the HIFU ablation in the treated lesion indicated a termination of tumor cell viability and a positive therapeutic outcome (Fig. 7).

It took many months to absorb the treated lesion. The anxiety regarding the persistence of a lump as the risk of recurrence is popular in patients, even if the absence of viable tumor cells by core biopsy. The disease- and recurrence-free survival rates are shown in Figure 8 In addition, 16 patients (94%) had a good or excellent cosmetic feedback, and 1 (6%) rated it as acceptable at the last follow-up.

No cell apoptosis was observed within the HIFU treatment region. Metastatic invasion, a complete ablation in removed axillary lymph nodes, was identified in H&E histology in some cases. Homogeneous coagulative necrosis has characteristics of tumor cell distortion, pyknotic nuclei, shrink of nuclei, cell debris, and disappearance (Fig. 9). There is also an obvious destruction on the non-neoplastic breast tissues and fat cells surrounding the necrosis. Granulation tissue with the presence of immature fibroblasts, inflammatory cells, and new capillaries were observed in the margin. Severely damaged tumor vessels illustrated the disappearance of endothelial cells nuclei, no distinction of cellular margins, and disrupted junctions between individual cells. Cellular discohesion, disruption of the smooth muscle tunica media, scattered intravascular thrombi occurred frequently in the treated vessels.

The treated breast cancer cells illustrated subcellular damage, destruction of plasma membrane, intracytoplasmic organelles, and nucleonic membrane at high magnification. Although the normal appearance of cancer cells after the ablation, presence of some vacuoles in the cytoplasm, cell membrane’s disintegration, and no identification of organelle structures suggest an irreversible cell
Table I. Percentage of reduction in tumor volume.

<table>
<thead>
<tr>
<th></th>
<th>3 mo</th>
<th>6 mo</th>
<th>12 mo</th>
<th>24 mo</th>
<th>36 mo</th>
<th>48 mo</th>
<th>60 mo</th>
</tr>
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<tbody>
<tr>
<td>Number of patients</td>
<td>22</td>
<td>22</td>
<td>21</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Average reduction</td>
<td>8.2 ± 6.1</td>
<td>26.7 ± 12.2</td>
<td>45.2 ± 22.1</td>
<td>72.3 ± 22.1</td>
<td>80.3 ± 38.2</td>
<td>87.3 ± 42.3</td>
<td>90.4 ± 49.1</td>
</tr>
<tr>
<td>Reduction range</td>
<td>2.5–18.7</td>
<td>19.2–49.1</td>
<td>25.2–70.1</td>
<td>50.2–82.3</td>
<td>58.1–90.6</td>
<td>70.3–92.1</td>
<td>80.5–96.3</td>
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Fig. 5. Contrast-enhanced MRI of a breast cancer (arrowed) (a) before and (b) 1 week after HIFU ablation with the lack of contrast uptake in the treatment region, including tumor and a 1.5–2.0 cm margin surrounding it. Oedema is observed in the surrounding mammary tissue.

Fig. 6. Sonography of a breast cancer (a) before (b) 6 months, and (c) 12 months after HIFU ablation with an obvious reduction of tumor size and an absence of blood supply (arrowhead).

Fig. 7. Tc-99m sestamibi SPECT of (a) two breast lesions (arrows) before HIFU and (b) disappearance of the radioisotope uptake in the treatment region (the lesions and 1.5–2.0 cm marginal breast tissue surrounding it) one month after HIFU.
death (Figs. 10(B and C)). The nuclear membranes disappeared, and chromatin was clumped at the periphery of the nuclei in the treated tumor cells. In the peripheral region, the treated breast cancer had an unrecognized amorphous electron-dense material (Fig. 10(A)).

Cancer had multiple small foci of tumor cells exhibiting cytoplasm reactivity. Interestingly, the preserved and damaged cellular structure of breast cancer showed intense cytoplasmic positivity simultaneously. Cytological enzyme activity (i.e., NADH-diaphorase stain), subside immediately on cell death, is used to identify acute cell viability more accurately and objectively than H&E staining changes in the cellular structure. No histochemical staining of pockets or foci of viable tumor cells was found in the both peripheral and central treatment region (Fig. 11). VEGF expression in breast cancer may stimulate tumor growth, angiogenesis, and metastases. However, CA15-3– and VEGF-positive expression was measured in only 52% and 30% patients receiving HIFU treatment, respectively.

![Fig. 8. Disease-free survival (DFS) and recurrence-free survival (RFS) rate for breast cancer patients underwent HIFU ablation.](image)

![Fig. 9. Histological section of (a) pyknotic nuclei of cancer cells, (b) disappearance of cancer cells nuclear, (c) denatured breast tissue, (d) coagulative necrosis of mammary gland tissue, (e) unviable fat cells, (f) new-growth granulation tissue in the marginal region of coagulative necrosis 9 days after HIFU ablation (H&E staining, 100×).](image)
Fig. 10. Cellular or subcellular structure of (A) breast cancer cells at the peripheral treatment region by HIFU with unrecognized amorphous electron-dense material and difficult identification on plasma membrane and intracytoplasmic organelles (6000×), (B) those at the central region with some vacuoles in cytoplasm, disintegrated cell membranes, and unclear organelle structures (3500×), (C) the discontinuity of nuclear membrane (arrow, 12,000×), and (D) a normal breast cancer (10,000×).

So histochemical analysis is not sensitive and reliable for confirming the generation of coagulative necrosis by HIFU.25

Proliferating cell nuclear antigen (PCNA), cell adhesion molecule CD44v6, and matrix metalloproteinase-9 (MMP-9) are molecular indicators as malignancy and nuclear immune-reaction of breast cancer cell in proliferation, invasion, and metastasis, respectively, using the biotin–streptavidin–peroxidase immunohistochemical technology (Fig. 12), whereas the stromal and inflammatory cells in the tumor were always negative. Positive cytoplasmic expressions of PCNA, erbB2 mRNA, CD44v6, and MMP-9 were found in 44%, 36%, 56%, and 60% of 25 breast cancer patients in the control group, respectively, but no staining was observed in the treatment region in the HIFU group.21

Furthermore, MRI-guided HIFU treatment for benign breast tumors was first carried out in 2001,26 and treatment success was defined as complete or partial lack of contrast uptake in the lesion on post-procedural T1w images was 8/11 (73%). The failure in 3 lesions may be due to low acoustic power and patient’s movement. 7 days after the session to determine the effectiveness of HIFU is recommended because some benign processes (i.e., oedema, fibrosis, necrosis, and inflammation) have similar characteristics in T1w images as those of a malignant process in that period. In addition, the first study on MRI-guided HIFU ablation on breast cancer as an adjunct to chemotherapy using tamoxifen citrate for elderly patients with high surgical risk was reported in 2003.27 19/24 (79%) patients had consistent negative biopsy
results after 1–2 HIFU sessions with only 1 case (5%) of recurrence (i.e., mucinous carcinoma). However, evaluating the clinical outcome of HIFU ablation for nonsurgical candidates should be careful, since other adjuvant modalities such as radiotherapy and/or chemotherapy may also have influence.

4. DISCUSSION

Development of breast imaging techniques and popularity in screening programs in the recent decades enable diagnosis of early stage breast cancer.6 Localized breast cancer is mostly treated using the gold protocol, breast conserving surgery with radiotherapy.6 Positive margins are found in 10–53% of the patients because of large tumor size, young age, positive axillary lymph node, and extended intraductal component.28 Although claimed as a low-morbidity procedure, breast-conserving surgery also accompanies complications, such as bleeding (2–10%), infection (1–20%), seroma formation (10–80%), and chronic incisional pain (20–30%).8,30

Compared to US imaging, enhanced MRI is more sensitive in the rapid assessment of HIFU-induced coagulative necrosis with no contrast enhancement in the ablation region and a thin peripheral rim surrounding it. SPCET also showed no radioisotope uptake in the treated tumor as an indicator of tumor death post-operatively. However, both MRI and sonography may underestimate tumor size, especially for an extensive ductal carcinoma in situ (DCIS) component, despite precise detection of a complete coagulation in the target by HIFU.31

If HIFU is implemented as an alternative to breast surgery, a reliable method must be available for tumor margin assessment and residual tumor diagnosis after ablation. MRI cannot delineate...
adequately the microscopic extent of tumor cells as histological examination. Most protocols are HIFU ablation followed by surgical resection, which allows pathological tissue examination to evaluate the HIFU outcome. Although no guidelines are established, the most popular and promising one is contrast-enhanced breast MRI and multiple large core-needle biopsies (LCNBs) for postoperative examination. If residual tumor or tumor recurrence in the lesion is found, the affected area will be re-treated. Applying both H&E and NADH histochemical staining is reliable in evaluating the therapeutic effects of HIFU.

One of important issues for HIFU success is selecting appropriate patients. A distance of at least 1 cm between the tumor and the skin (to avoid superficial burn), and between tumor and chest wall (to avoid thermal accumulation in the underlying ribs and lung) is required. Lying in the closed MRI magnet for 1–2 h may introduce physical and psychological problems, especially in anxious or claustrophobic patients. Large tumors (>5 cm) result in a significant increase of the treatment time and a decrease in the probability of complete tumor necrosis substantially. In addition, patients with extensive DCIS should be excluded. However, for non-surgical candidates with locally advanced breast cancer or metastases, the criterion of selecting patients are less important in the palliative HIFU ablation.
Metastases in regional axillary lymph nodes of breast cancer patients, which is determined by the minimally invasive sentinel lymph node biopsy (SLNB), is an important prognostic factor. HIFU might affect the accuracy of SLNB by obstructing or changing the anatomy of breast lymphatics or lymph drainage. Use of contrast agent and isotope in the diagnosis of the sentinel node could improve the overall success rate to 91%, which is comparable with that of SLNB. Further investigation is required to assess the ability to perform SLNB after HIFU ablation.

A palpable firm lump would persist or even become larger for a certain time after HIFU ablation, although the lesion would become benign eventually. The fear of local recurrence had an impact on the patient’s psychology, satisfaction, or cosmetic concern. As a result, some patients received a modified radical mastectomy afterwards. In addition, in the follow-up examination it is hard to distinguish the firm lump and local recurrence postoperatively.

Another clinical application is gene therapy, which requires precise control of transgene expression both temporally and spatially in the tumor cells. A heat-sensitive promoter (i.e., HSP70B) and a reporter gene, such as green fluorescent protein (GFP) or firefly luciferase (Fluc), were used in vivo to monitor the HIFU mediated transgene expression. Activation of a therapeutic transgene, such as tumor necrosis factor α or interleukin 12, during or after HIFU treatment will be investigated.

Since chemotherapy causes significant systemic toxicity, improvement of targeted drug delivery has a great impact in clinics. Heat-sensitive liposomes with the encapsulated chemotherapeutic drug with prolonged intra-vascular circulation time have already been developed and investigated. Because the permeability of malignant tumor vessels are high (neo-angiogenesis), liposomes will accumulate in the interstitial space in the tumor. Meanwhile, HIFU-induced hyperthermia and bubble cavitation will enhance both extravasation and drug release. However, a recent study of delivery of liposome-encapsulated doxorubicin by HIFU in a mouse breast cancer model did not achieve expected results.

Hyperthermia, raising the tumor temperature to 42–45 °C for 30–60 min, has been used together with radiotherapy or chemotherapy to induce the apoptosis of tumor cells via a multiple-session procedure since the 1980s. US can also induce apoptosis via either cavitation or thermal production. However, HIFU ablation can produce coagulative necrosis in the tumor via a single-session procedure without intervention of the others. Therefore, mechanisms and consequent pathological characteristics of HIFU and US hyperthermia are fundamentally different. There is no dependency of the HIFU effectiveness of the tissue type, so both breast cancer cells and normal tissue are destroyed simultaneously. Open surgery is still necessary to remove axillary lymph nodes after HIFU therapy, which reduces the non-invasiveness of this modality. Therefore, HIFU must be implemented together with conventional therapies (i.e., chemotherapy, radiotherapy, and endocrine therapy) in order to kill micro-satellite metastasis, reduce local recurrence, and prevent the risk of relapse for the optimal therapeutic outcome.

Overall, this non-invasive technology has more acceptances by patients with the consideration of psychology and cosmetics than conventional therapies. Preliminary results are very encouraging, but it is still too early to conclude the long-term efficacy and role of HIFU in the treatment of breast cancer because of scant evidence for this fairly new technique.

5. TUMOR VESSEL DESTRUCTION

Malignant tumors has several inherent characteristics in the histological structure and circulatory function of microvasculature that are invulnerable to hyperthermia damage, such as thin-walled lumen for easy compression, lack of perivascular smooth muscle with much less vasmotor control, large inter-capillary distance, increased permeability, arteriovenous shunts, heterogeneous blood flow with consequent foci of relative hypoxia and acidosis. Except the coagulation production in HIFU ablation for an irreversible cell death, the thermal and mechanical damage to small vessels and cessation of the blood circulation in the tumor would cause deprivation of nutrition and oxygen for the neoplastic cells, which accounted partially for the formation of tissue necrosis. An abrupt interruption and immediate decline of blood flow within the treated tumor vessels could be measured quantitatively by color Doppler imaging (Fig. 13).

Digital subtraction angiography (DSA) indicated a remarkable decrease of hypervascularity and almost disappearance of tumor vascularity and stain in the patients following HIFU (Fig. 14). HIFU induced homogeneous coagulative necrosis of tumor tissue as well as of small arteries and veins in the tumor. Vascular elasticity and collagen fibrin in Victoria blue and Ponceau’s histochemical staining were found to collapse and disrupt significantly in the treatment region, which indicated that the severe destruction of the HIFU ablation on the tumor’s vascular wall (Fig. 15). Damaged endothelial walls might accumulate collagen fibrin, activate platelet, and eventually cause the formation of thrombosis. Significant vessel rupture, vascular disruption, and vessel occlusion have been demonstrated in various HIFU experimental conditions. The vascular destruction effects depended on the HIFU operation parameters (i.e., frequency, intensity, duty cycle, and duration) and the vessel characteristics (i.e., size, elasticity, and blood flow).

The failure to early detect viable tumor after HIFU ablation usually leads to the local recurrence and remote metastases. Therefore, CT or MRI is routinely used to evaluate the therapeutic outcome and the extent of coagulative necrosis. The pathologic characteristics of the obvious damage in small blood vessels and capillaries correlated with the cessation of blood circulation in the treated tumor in post-procedural imaging. However, sometimes the imaging quality is not sufficiently high to identify the therapeutic response. Due to its wide availability, real-time capability, flexibility, and low cost, color Doppler US is common in assessing the vascularity changes. If the blood supply to the tumor is poor, the US imaging is not sensitive in detecting such a destructive response. I.V. administration of US contrast agents (microbubbles) could improve the evaluation accuracy of vascularity.

6. TUMOR ANTIGEN AND IMMUNE RESPONSE

Tumors can escape the immune surveillance by interfering with the migration of dendritic cells (DCs) and by failing to provide the necessary activation signals as well as by secreting active factors that inhibit the differentiation and functions of DCs. Thus,
Fig. 13. Hypoechoic mass with numerous blood flow in the tumor before HIFU and (b) no flow in the treatment region 2 weeks after HIFU for a thigh soft tissue sarcoma color Doppler US images.

Fig. 14. (a) Persistence of tumor vascularity and capillary stain within distal femur osteosarcoma and (b) complete disappearance of tumor vascularity and stain within the tumor 3 months after HIFU ablation in DSA.

Fig. 15. (a) Normal structure of elasticity fibrin (solid arrow) and collagen fibrin (open arrow) within the vascular wall in the untreated breast and (b) significant collapse and disruption of them in the HIFU-treated one (Victoria blue and Ponceau’s histochemical staining, 400×).
the immune system cannot control the cancer growth and prevent local recurrence and metastasis. DCs can infiltrate primary breast cancer, but their number and distribution vary considerably between patients.56,57 The tumor-infiltrating DCs are neither mature nor activated,58 and the phenotypic and functional DCs are severely impaired by tumor-secreted immunosuppressive cytokines,59,60 which may lead to the dysfunction of DCs and make breast cancer weakly immunogenic and, therefore, a poor candidate for immunotherapy.61

Host immune system protects abnormal cell growth and lymphocyte (i.e., CD3+ T lymphocyte in the peripheral circulation) mediated immunity prevents the growth of primary tumors and subsequent metastases.62 The tumor progression the micrometastatic, and long-term tumor resistance result from a failure of the immune system either to recognize or to mediate tumor destruction, which could be treated by immunotherapy to not only destroy local tumors but also restore and activate a systemic antitumor immunity. In order to achieve antitumor immunity of the host, tumor antigens that can elicit immune responses specific to the tumor cells must be expressed.

It has been demonstrated the function of lymphocyte-mediated cellular immunity is suppressed after both surgical procedures,63,64 and local radio-frequency ablation.65 Tumor debris enhanced inflammatory and immune reactions in the peripheral coagulation zone to serve as a potential antigen source,66 which may result in longer survival time.67

Antigen presenting cells (APCs) play a critical role in immune responses, and can infiltrate local tumors and present tumor antigens to naive T lymphocytes. Two factors are essential for APCs to initiate an efficient antitumor response: the activation signals delivered directly or indirectly by tumor cells, and tumor-infiltrating APCs activated locally. Activating signals can induce the progression of infiltrating APCs from an immature to a mature stage. Afterwards, APCs increase the expression of co-stimulatory molecules (i.e., CD80 and CD86) and become efficient in a process of cross-priming T cells.68 However, the absence or blockade of these co-stimulatory molecules impairs tumor antigen-specific immune responses, indicating the requirement of APCs activation in antitumor immunity.69 The transfer of tumor antigens from APCs to T lymphocytes is critical in initiating lymphocyte-mediated immunity. Macrophages, B lymphocytes, and DCs are the most potent APCs in the uptake, processing, and presentation of tumor antigens.70

HIFU ablation can denature the secretion of immunosuppressive protein constituents originating from tumor cells70 and change the unfolding of proteins from the native state to a more random state of lower organization,71 which leads to either loss or preservation of antigenic determinants due to both thermal and cavitation. Large amounts of tumor debris containing various tumor antigens can be exposed in situ and reabsorbed later. The most striking change in the tumor debris was the up-regulation of heat shock proteins (HSPs),72–75 whose tumor peptide complex is taken up by APCs and presented HSPs directly to tumor-specific T-cells with high efficiency and enhanced tumor immunogenicity.76,77 A variety of molecules expressed in breast cancer cells (i.e., tumor antigens and HSP70) were stained using...
biotin-streptavidin-peroxidase immune-histochemical technology in all breast specimens. The ablated region had the cellular structure of thermal fixation and large amounts of positively stained tumor cells with pyknotic nuclei (Fig. 16). In contrast, typical characteristics of normal tissue were illustrated in the peripheral region. Because of unique tumor antigens by random mutations of cancer cells in each patient, personalized HSP vaccination does not require the identification of the unique antigens.

Immune function was determined by the ratio of T-cell helper/suppressor (CD4/CD8). A significant decrease and increase of average CD4+ level \( (p < 0.01) \) and CD4/CD8 ratio \( (p < 0.05) \) in peripheral blood was observed before and after (7–10 d) HIFU treatment, respectively (Fig. 17).

In the control group, APCs were observed in all tumor specimens, although with a high occurring frequency in the periphery than in the center of the breast cancers. S-100+ cells were distributed sparsely, and their cytoplasmic processes extended between the cancer cells (Fig. 18). However, the infiltration of diffusely scattered CD68+ and CD20+ was more heterogeneous. Almost no immune-stained cells were identified in the center of the tumors because of the death of both APCs and cancer cells and no immediate wound-healing after HIFU ablation. However, APCs were observed in the granulation tissue along the margins of ablation together with immature fibroblasts, new capillaries, and other inflammatory cells (Fig. 18). Among them, CD68+ and CD20+ cells were usually in small clusters, whereas S-100+ cells had a scattered distribution. The number of

![Fig. 18. Positive expression (brown) of tumor-infiltrating (A) S-100 cells, (C) CD68 cells in a small cluster, (E) and CD20 cells within a cluster of lymphocytes at the margin of HIFU ablation, and the corresponding ones (B), (D), and (F) within breast cancer cells in the control group (streptavidin-peroxidase immune-histochemical staining, 200×).](image1)

![Fig. 19. Positive expression of the tumor infiltrating S-100, CD68, and CD20 cells at the peripheral region of tumor in both groups (*significant statistical difference between the control and HIFU groups).](image2)
CD68+ and CD20+ cells was higher than that of S-100+ cells without appreciable difference of the infiltration among them.\(^7\) There is a statistical increase in the number of the APCs after HIFU ablation, S-100+ \((p < 0.01)\), CD68+ \((p < 0.006)\) and CD20+ cells \((p < 0.007)\) between the control and HIFU groups, respectively (Fig. 19).

In the double-immunostained sections, S-100+ and CD68+ cells were confirmed by intense cytoplasmic staining (purple), and their expressing HLA-DR, CD80, and CD86 were confirmed by both cytoplasmic staining (purple) and membrane staining (red), respectively. Coincident staining resulted in a positive reaction of black coloration in the double-stained positive cells (Fig. 20). The infiltration of the double-labeled cells was similar to those on the single-stained sections, but in a smaller proportion.\(^7\)

In summary, the immunologic abnormality and the lack of a host antitumor response could be reversed after the HIFU ablation on primary cancer,\(^7\) which suggests that the HIFU-induced activation of T lymphocyte-mediated antitumor immunity. The factors and mechanism involved in this phenomenon are very complicated and not fully understood. One possible mechanism is that suppression effects on anti-immunity by tumor cells are partially or completely restored after tumor destruction. Acoustic radiation and bubble cavitation in the high-intensity acoustic field may also alter the tumor structures and impair the function of tumor-specific T cells.\(^7\) It is shown that in vitro DCs and macrophages exposed to the supernatants of HIFU-treated tumor cells had a greater expression of co-stimulatory molecules with more IL-12 and TNF-alpha from DCs and macrophages \((p < 0.01)\), respectively.\(^7\) It suggests that HIFU ablation may not only cause significant infiltration of APCs in the treated tumor, but also induce the tumor infiltrating DCs from an immature to a mature stage. Therefore, the HIFU-induced immune response will be investigated further as an alternative modality in treating cancers.

7. CONCLUSION

Multiple phase I and II studies have already proven the feasibility and safety of MRI- and US-guided HIFU ablation of small (<2 cm) solitary breast cancer with success rate of 20%–100%, depending on system configuration, guidance technique, ablation protocol, and patient selection criteria. Although the preliminary results for localized breast cancer are promising, the data are not sufficient to justify a random trial and compare with breast conserving surgery. To date, MRI has better performance in accurate targeting, delineation, and thermal monitoring for breast tumor during the procedure as well as detecting residual
disease post-operatively. A large prospective study is required to assess the therapeutic efficacy of this novel technology as well as to standardize technique and formulate a procedure guideline. In addition, HIFU-mediated anti-tumor immune response, localized transgene expression, and drugs delivery enhancement could be explored further.

Noninvasiveness of HIFU makes it appropriate for elderly women with early stage breast cancer. HIFU may also serve as a salvage method to eradicate small primary, residual or locally recurrent tumors that persist after the completion of breast-conservation surgery. Finally, treatment of breast fibroadenoma, a common benign tumor in young women, in outpatient settings by HIFU is of possible. HIFU ablation should be carried out with precise information not only of the lesion’s number and location, but also of the biological characteristics and natural history of the tumor. The following treatment of breast cancer should be multidisciplinary, including surgery, radiotherapy, chemotherapy, and/or tamoxifen.

The lack of pathological examination on tumor margin directly and accurately is a major obstacle for thermal ablation approach. Ideally, HIFU should give almost the same results as surgical excision. There are many technical limitations to be overcome before wide acceptance, such as the lack of controlling the focal spot positions, precise target definition, and thermometry. Although recent and sparse data are very encouraging, further prospective and randomized trials worldwide are of importance to evaluate the long-term efficiency and efficacy, cosmetic outcome, and cost-effectiveness of HIFU treatment for breast cancer patient in early-stage. After resolving all of these issues, this noninvasive technology can be considered as candidate for conventional therapy for clinical application.

References and Notes