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REVIEW

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Low-frequency HIFU induced cancer immunotherapy: tempting challenges and potential opportunities

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ABSTRACT Immunotherapy is playing an increasingly important role in the treatment of tumors. Different from the traditional direct killing or excision therapies, immunotherapy depends on autologous immunity to kill tumor cells and tissues by activating or enhancing the body's immune system. Large numbers of recent studies suggest that low-frequency HIFU can not only enhance the intensity of the body's anti-cancer immune response, but also improve the efficiency of immunotherapy drug delivery to strengthen the effects of tumor immunotherapy. The focused ultrasound (FUS) destructs the tumor and simultaneously generates tumor debris and tumor-associated antigens, which enhances the immunogenicity of the tumor and stimulates the immune cells, inducing the body's immune response. Microbubbles are clinically used as a contrast. As a matter of fact, the addition of microbubbles can reinforce the destructive effect of FUS on the tumor and activate a stronger immune response. The combined application of ultrasound and microbubbles can more effectively open the blood brain barrier (BBB), which is beneficial to improving the intake of immune cells or immunotherapy drugs and exerting a positive influence in the lesion area. Currently, microbubbles and nanoparticles are commonly used as gene and drug carriers. Using ultrasound, the immune-related gene or antigen delivery itself can enhance the immune response and improve the efficacy of the immunotherapy.

KEYWORDS Immunotherapy; low-frequency HIFU; biological effect; immune response; combination therapy

Introduction

Malignant tumors are one of the main diseases that seriously threaten human life. The therapeutic regimens commonly used in clinic include surgery excision, radiotherapy, and chemotherapy. As several traditional treatment means, they can treat the primary cancers very well although their drawbacks, such as uncontrolled recurrence and metastasis, cannot be ignored in the practical application, so new cancer therapeutic methods need to be developed urgently[1]. Immunotherapy, a new type of cancer treatment, does not kill the cancer cells directly. By activating or enhancing the human immune system, the cancer immunotherapy eradicates the cancer cells or tissues depending on autoimmune function[2]. The target of this method aims at the autologous immune system instead of the cancer cells or tissues[3]. Cancer immunotherapy has received wide attention because of its great clinical application value[4].

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The functions of the immune system are mainly manifested in three aspects: defense, stabilization and immune surveillance. Once these functions are out of balance, immunopathological reactions occur[5]. Immunity of the body can be roughly divided into two kinds: specific immunity and non-specific immunity. Non-specific components need not be exposed beforehand and can respond immediately, effectively preventing the invasion of various pathogens. Specific immunity is developed in the lifetime of the body, and is specific to a certain pathogen[6]. Dendritic cells (DCs) are the most powerful antigen presenting cells and can efficiently absorb, process and present antigens. Immature DCs have strong migration ability. Mature DCs are at the center of initiation, regulation and maintenance of immune response, and can activate the initial T cells effectively[7]. An effective immune response includes three procedures[8]: (1) The DCs capture tumor antigens and process them inside acquiring "mature signals" to activate the immune response for the antigens. (2) DCs present antigenic information to the immature T cells in lymphoid tissue and make them activated, which initiates the specific immunity of the human body. If the DCs presenting antigens are activated by immature signals, the T cells will be induced to create tolerance and consequently resist the

immune response. (3) The activated T cells infiltrate the cancer tissue, specifically recognize and kill cancer cells. However, tumor cells possess the nature of immune escape, which makes them avoid the recognition and attack from the immune system by multiple mechanisms, so it is very difficult to eradicate the tumor cells or tissues *via* the autoimmune system of the human body. Eradication of tumors by means of stimulating the autoimmune system is very promising for anticancer treatment[9,10]. Currently, tumor immunotherapy mainly includes the following types: enhancing a specific immune system[11], tumor vaccine[12,13], and adoptive cell transfer (ACT)[14].

Ultrasound (US) is defined as a kind of mechanical wave with a frequency over 20 kHz and cannot be detected by the human ear. Aside from medical imaging, US also performed well in the treatment field[15]. In recent years, many studies indicate that US can be used to promote the anti-cancer immune response of the human body[16,17]. Focused ultrasound (FUS) can not only create thermal effects but also create mechanical effects or cavitation effects[18]. The mechanical effects or cavitation effects can increase the anti-cancer immune response of the host body[19]. Theoretically, these effects can be classified by various US parameters. Cavitation effect, as one of the main mechanical effects, usually occurs at low frequency, high intensity and low duty cycle[20]. The thermal effects of US often occur at high to moderate intensity and longer duty cycles, and they are generally used in combination with thermosensitive formulations[21]. Genes and antigens are delivered into the cancer cells or tissues adopting US, which can also activate the anti-cancer immune response[20,22]. The force exerted on the cell membrane when the microbubble is broken by US directly delivers the substance to the cell, evading each kind of natural barrier[23]. By this means, one can deliver the cancer antigen and the antigen-encoding gene to immune cells, and the gene that stimulates the immune response can be also delivered to the cancer cells[24,25]. In this paper, we focused on activation of the anti-tumor immune response using low-frequency high-intensity focused ultrasound (HIFU).

Low-frequency HIFU

Biological effect of low-frequency HIFU

US is generally considered safe for imaging in vivo except for two side effects[26-28]: thermal and mechanical effects (including sonoporation). However, these two adverse factors for medical imaging are very important to treatment using US. Thermal effects depend on the absorption and accumulation of US energy. The intensity of US, irradiation time, and biological properties of tissue are the main three factors that determines the amount of heat[29]. The dose of US has a very strong relevance with thermal and mechanical effects. At a low intensity, the ratio of apoptosis to lysis is high, and with the increasing of intensity, lysis becomes predominant over apoptosis and directly causes cell to death. More effective induction of apoptosis is obtained if paused modulation is used with a longer pause than the irradiation time[30,31]. Accounting for the degree of membrane damage and the capacity of repairing the damage, the death of a cell can be divided into three modes, instant lysis, necrosis, and apoptosis[32,33]. **Figure 1** shows the correlation between the degree of membrane damage caused by US and the corresponding cell death mode[34,35]. Although some of the damaged cells can successfully self-repair and eventually survive, the process of US irradiation will speed their apoptosis or necrosis[36].

Cavitation effect

The mutual effects between microbubbles and US can easily lead to the cavitation effect[37]. Microbubbles oscillate symmetrically and linearly at a low powered US field, which implies an opposite tendency of the expansion and compression of a microbubble[38]. According to the different dynamic behaviors of bubbles, cavitation effects of an US wave can be divided into stable cavitation and inertial cavitation[39,40]. If the sound intensity of US is weak, the cavitation nucleus is enlarged in the negative pressure stage of sound pressure, and then compressed and reduced in the positive pressure stage[41]. In other words, the bubble vibrates radially with the balanced radius of sound frequency, which is called steady state cavitation. When the bubbles are compressed under greater pressure to a certain degree, the density of mixed gas inside the bubbles will increase, and it is difficult for the bubbles to continue being compressed[42]. However, because of the inertial push from the surrounding fluid, the increasing speed of pressure on the bubble wall is still very fast, even surpassing the compression speed of gas

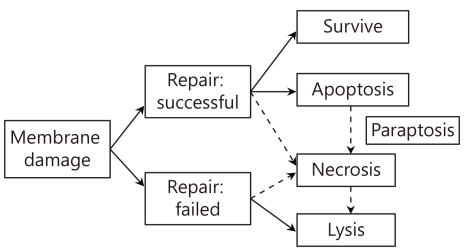

Figure 1 Schematic presentation shows the correlation of membrane damage, repair or not, and mode of cell death.

inside the bubble. Under such a condition, bubbles will collapse, and the intensity is determined by the inertia of inward-pushing fluid[43-45]. That is defined as inertial cavitation. The cavitation effect induced by ultrasound-mediated microbubble destruction can directly damage tumor cells and cause obvious ultrastructural changes in tumor neovascularization, which can lead to mitochondrial swelling, myeloid degeneration, and cytoplasmic cavitation of endothelial cells promoting apoptosis[46,47]. If the cavitation effect is very strong, the walls of small tumor blood vessels can be damaged, which will activate endogenous or exogenous coagulation, and induce thrombosis in blood vessels leading to large-area capillary embolization, blocking the nutritional supply of cancerous tissue cells, and then causing the necrosis of local tumor cell[48]. Lethal cavitation effects can directly lead to lysis and death of tumor cell. All these provide a theoretical basis for the direct treatments of tumor with ultrasonic microbubbles[49-51].

Medical application of low-frequency HIFU

Low-frequency HIFU is widely used clinically for drug delivery, fractured bone and cartilage, nerve stimulation, inflamed tendons, wound healing and ligaments repairing[52,53]. Compared with the thermal therapy of high-energy US, the non-thermal effects of low-frequency HIFU mainly display in the mechanical stimulation induced by microbubbles, microjets, cavitation and acoustic steaming[54-57]. In fact, low-frequency FUS can reach deep into the body, which allows precise local treatment and avoids possible disadvantageous side effects to surrounding healthy tissues[58]. In the nanomedicine field, US stimulus has shown great application prospects such as strengthening extravasation of nanoparticles through blood capillaries, increasing cell membrane permeation, inducing an anti-tumor immunity and so on[59,60]. Immunotherapy can be classified into active immunotherapy and passive immunotherapy[61]. Both these two disease treatment strategies can be reinforced by low-frequency HIFU[62].

The blood brain barrier (BBB) is a protective barrier system between blood and brain tissue, which is composed of endothelial cells, basal membrane, and glial cell podocytes of brain capillaries[63]. The BBB allows nutrients needed by brain tissue to pass through, avoids causing brain damage by effectively preventing some foreign bodies and large molecules from passing through, and stopping harmful substances in the blood from invading the brain[64]. Because of the filtering effect, the BBB affects the deliver ability of drugs and antibodies to brain tissue in almost all intracranial neurological diseases, which limits many drugs and antibodies to low concentrations when entering the brain from the blood, making it difficult to apply the due effect[65,66]. Currently, there are three main strategies for delivering drugs to the brain[67]. One is to compound new small molecules that can reach the brain tissue, but only a few diseases can be treated with small molecules. The second is to deliver drugs to the brain by using invasive catheters, but a local puncture can easily damage brain tissue. Third, noninvasive and reversible opening of the BBB, and such an idea has attracted increasing attention. It has been one of the research focuses for scientists to open the BBB by combination of FUS and MB[68]. The mechanism can be explained as follows[69,70]: (1) US waves cause microbubbles to expand and collide in capillaries, and the larger microbubbles expand and fill the capillary lumens, leading to the mechanical dilation of blood vessels, which results in the opening of tight connections. (2) Pressure changes in capillaries induce biochemical reactions that trigger the opening of the BBB. (3) Microbubble vibration can reduce local blood flow and lead to transient ischemia, triggering the opening of the BBB. (4) US irradiation causes the burst of micro-bubbles, resulting in local high-speed turbulence and jet flow, and these mechanical effects also participate in the opening of the BBB[71]. **Figure 2** shows the FUS-induced BBB opening and its potential effect in CNS immune modulation and immunotherapy[72]. So far, there have been many clinical reports about using low-frequency HIFU to open the BBB, such as the delivery of tumor-targeted drugs, gene vectors and analgesics[73,74].

Low-frequency HIFU induced tumor immune response

The primary mechanisms of low-frequency HIFU mediated immune response are as follow[75,76]: (1) After the irradiation of low-frequency HIFU, both the tumor debris and the released relating cancer antigens can work as a cancer vaccine, enhancing the immunogenicity. (2) The treatment of low-frequency HIFU on the tumor lesion can induce Th1 reaction, which leads to significant changes of cellular immunity, strengthening the activity of DC and cytotoxic lymphocytes. (3) The treatment of low-frequency HIFU can balance the immunosuppressive action induced by the tumor microenvironment. The three effectors above can effectively stimulate the anti-cancer immune response of the human body. Currently, there is abundant literature for preparing tumor lysates from tumor samples aiming to activate the anti-tumor response[77]. The tumor lysates are loaded on

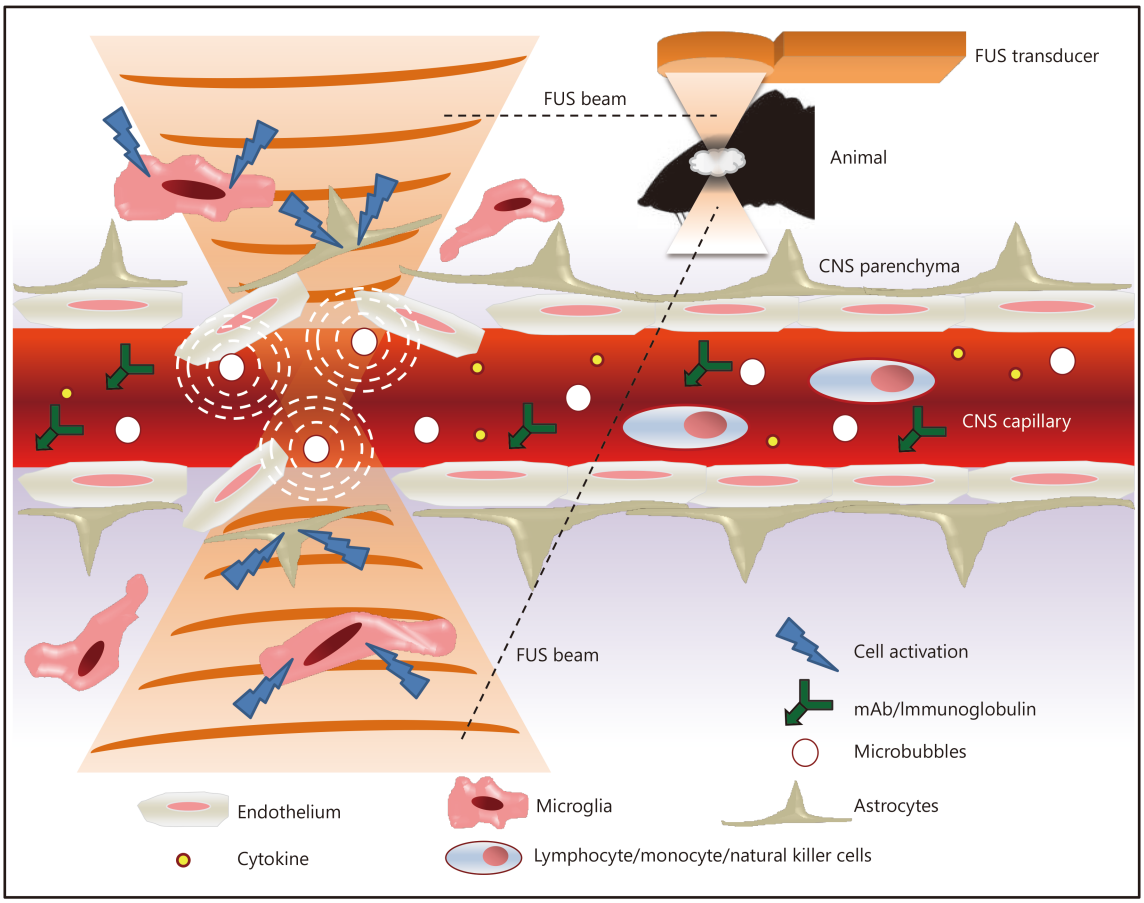

Figure 2 Schematic showing FUS-induced BBB opening with its potential effect in CNS immune modulation and immunotherapy. Adapted from reference 72.

cytotoxic T lymphocytes (CTLs), which will enhance the killing activity aiming to cancer cells. Many literatures have given the detection and analysis methods of related immune parameters, such as cytokine detection, chemokine detection and so on[78].

Compared with high-frequency HIFU, low-frequency HIFU can reserve the antigens more efficiently, stimulate the DCs' infiltration and maturity in situ, which triggers a stronger immune response[79]. In addition, the different scanning ways of low-frequency HIFU also affect the immunotherapy effect[78]. In diseased tissue, the sparse scanning mode can reserve antigens better, and be more effective than the intensive scanning mode[59,80]. Yang et al.[81] discovered very early that FUS can stimulate the anti-cancer immune response. They treated neuroblastoma C1300 mice with HIFU, and the same cancer cells were inoculated again in the mice after the ablation of cancer tissue. Comparing with mice that were not inoculated for cancer cells in the initial stage or the mice that were not treated with HIFU, they found that the proliferation rate of the re-inoculated tumor cell after HIFU treatment is significantly decreased. Many subsequent studies also indicated that the tumor debris treated with FUS can induce the tumor specific immune response, so the tumor debris after FUS treatment can be taken as effective anti-tumor vaccine[56,60,81-88]. A summary of the HIFU anti-cancer response is displayed in **Figure 3**[89].

Studies showed that the adoptive transfer of immune cells activated with low-frequency HIFU also has very good effect[90]. After treating H22 cancer-bearing mice with low-frequency HIFU for 14 days, the T cells were taken out and then adoptively transferred into other mice bearing H22 cancer[91]. The experimental data indicates that CD3+, CD4+, the ratio of CD4+/CD8+, CTL cytotoxicity, and the secretion of IFN-γ and IFN-α of the mice after low-frequency HIFU treatment all increase significantly. After transferring the activated T lymphocytes to the cancer-bearing mice, both the tumor infiltration T lymphocytes and IFN-γ secretory cells increased significantly. **Table 1** shows the overview of recent clinical research on immune effects after the irradiation of

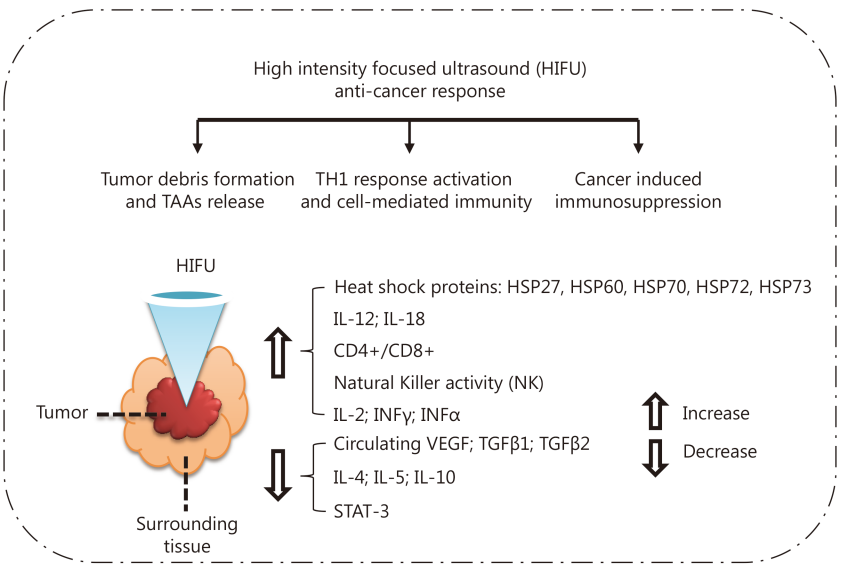

Figure 3 Summary of HIFU-induced anti-cancer response, in which STAT means signal transducer and activator of transcription, and TAA means tumor-associated antigens. Adapted from reference 89.

FUS[92-99].

Combined treatment strategies

Immunotherapy assisted by microbubbles and low-frequency HIFU

Cavitation effect is the important physical foundation of US-enhanced drug delivery and US-guided gene transfection[100]. One can deliver drugs or transfect genes by using the cavitation effect of US, and assist the tumor cellular immunotherapy[101]. Microbubbles are commonly used as gene or drug barriers. The US microbubble contrast agent can enhance the cavitation effect, improve drug delivery efficiency, and increase local drug concentration when the drug is loaded on the microbubbles[102]. The simplest method using microbubbles to deliver the active substance is co-injection[103]. Microbubbles and the active substance are mixed in some solution in vitro, and US irradiation promotes the delivery in vivo. However, the potential problem of co-injection is that the distribution of microbubbles and active substance in vivo is not exactly the same, which leads to the decrease of drug availability[104]. A better method is to conjugate the microbubbles with the active substance, and this method can ensure the same bio-distribution of two conjugating objects, improve the local concentration of the active substance, and decrease drug dosage[105]. The combination of targeted microbubbles and US afford a more complex and efficient delivery system. Such a therapeutic system possesses site specificity and cell specificity, which activates the immune system more efficiently[104,106]. **Figure 4** shows the use of US with mRNA-loaded microbubbles, in which the mRNA-loaded microbubbles implode upon exposure to US and sonoporate the DCs[107]. As a result, both antigen and DC-modulating proteins are produced by DCs, which can lead to antigen presentation and T-cell activation. Many of the studies on microbubbles for drug and gene delivery have used commercially available US imaging contrast agents or similar bubbles equipped with targeting ligands on the surface or bubbles complexed with active substances[108,109].

Even without any active agent, microbubbles still possess potential as immune response triggers[110]. In recent research, the effect of SonoVue microbubbles was examined in combination with focused US on solid CT-26 tumors in mice[111]. Intravenous injection of microbubbles came first, and then irradiation of US on the tumor followed immediately. Compared with results treated with only US, the combined therapeutic strategy distinctly decreased the tumor growth. Infiltration of immune cells increased in the tumor tissue, in which the CD8+CTL and CD4+non-Treg levels are included. As for the immune cells, microbubbles have also been commonly used to promote the permeability of the BBB under the irradiation of US[112]. And the natural killer cells moving through the BBB come at a much higher extent than if only US was used[113,114].

Microbubbles, usually taken as carriers of delivering genes and antigens in immunotherapy, also have some

Table 1 Overview of described immune effects after irradiation of FUS in clinical studies

Year	Authors	Patient information	Ultrasound parameters	Key observations
1998	Maders-bacher et al.	5 patients with clinically localized prostate cancer	Frequency: 4.0 MHz Focal length: 2.5, 3.0, 3.5, 4.0 cm Acoustic intensity: 1,260−2,200 W/cm^2 Exposure time: 4s on followed by 12s off for re-positioning.	Consistent HSP-27 expression was observed at the border zone of thermonecrosis *in vivo*, with highest levels occurring at 2-3 h following transrectal HIFU
2004	Wu et al.	16 patients with solid malignancies (osteosarcoma, hepatocellular carcinoma, renal cell carcinoma	Frequency: 0.8 MHz Focal length: 135 mm Acoustic intensity: 5,000−20,000 W/cm^2 Exposure time: variable Therapeutic time: 2.5−8 h (median: 5.2 h)	Both the circulating CD4+ lymphocytes and the ratio of CD4+/CD8+ increased in patients after receiving HIFU
2004	Kramer et al.	6 patients with prostate cancer	Frequency: 4 MHz Focal length: not provided Acoustic intensity: 1,260−2,000 W/cm^2 Exposure: 4 s per location	A significant upregulation of HSP-72 and HSP-73 at the border lesion after HIFU treatment in prostate cancer patients
2007	Wu et al.	23 patients with biopsy-proven breast cancer	Frequency: 1.6 MHz Focal length: 90 mm, Acoustic intensity: 5,000−15,000 W/cm^2 Exposure time: 45-150 mins (median: 1.3 h)	All tumors treated with HIFU stained positive for epithelial membrane antigen and HSP70. No tumors treated with HIFU stained positive for CD44v6, MMP9, or PCNA
2008	Zhou et al.	15 patients with solid malignancies	Frequency: 0.8 MHz Focal length: not provided Acoustic intensity: 5,000−20,000 W/cm^2 Exposure time: 0.78-3.62 h (mean: 2.74 h)	Patients exposed to complete or partial HIFU ablation experienced a reduction in serum immunosuppressive cytokine expression levels, with nonmetastatic patients experiencing lower expression levels as compared with metastatic patients. VEGF, TGF-β1, and TGF-β2 were significantly reduced following HIFU treatment
2009	Lu et al.	48 female patients with biopsy-proven breast cancer	Frequency: 1.6 MHz Focal length: not provided Acoustic intensity: 5,000−15,000 W/cm^2 Exposure time: 45-150 mins (mean: 1.3 h)	Neoplasms treated with HIFU expressed elevated NK cells as well as CD3+, CD4+, CD8+, and B lymphocytes in the ablated periphery TILs positive for granzyme, FasL, and perforin were also greater in response to HIFU as compared with untreated control tumors
2009	Xu et al.	23 female patients with biopsy-proven breast cancer	Frequency: 1.6 MHz Focal length: not provided Acoustic intensity: 5,000−15,000 W/cm^2 Exposure time: 45-150 min total time	A significant increase in infiltration and activation of macrophages and DCs in HIFU-treated tumors, compared to controls
2013	Wang et al.	120 patients with uterine fibroids (subserosal, intramural myomas, infertility, recurrent pregnancy loss)	Frequency: 0.8 MHz Focal length: not provided Acoustic intensity: 400 W/cm^2 Exposure time: 24 h or 72 h	120 patients were divided into two groups, HIFU group and myomectomy group. Serum levels of IL-6 and IL-10 increased after treatment in both groups. Peak IL-6 and IL-10 levels were significantly lower in the HIFU group than in the myomectomy group. In contrast, IL-2 level decreased significantly in the myomectomy group compared to the HIFU group at 24 h post-operation

disadvantages[115,116]. Firstly, the diameter of microbubbles is generally in the range 2−5 μm, the tumor tissue can be penetrated effectively without the irradiation of US. Secondly, the half-life period of microbubbles is relatively

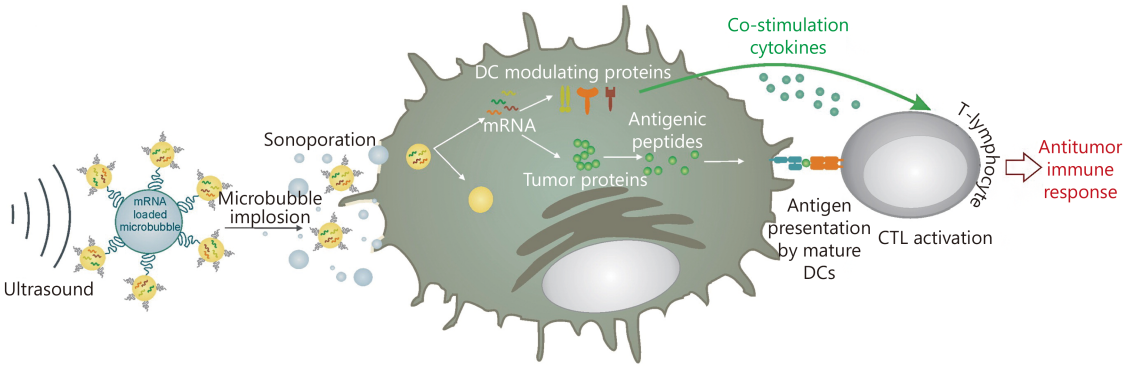

Figure 4 Schematic of the use of US with mRNA-loaded microbubbles. Adapted from reference 107.

short in vivo, which is not beneficial to gene or drug delivery. One other potential problem is their complex preparation method, and much pretreatment is needed before use. The disadvantages above have limited the application of microbubbles in immunotherapy to a certain extent[117].

Nanoparticles for low-frequency HIFU cancer immunotherapy

Nanoparticles have high surface area to volume ratio and advantageous delivery kinetics, and can be well used in clinical diagnosis and treatment including tumor immunotherapy according to their unique physical and chemical properties[118]. The size of nanoparticles are ranging from 1nm to 100nm[119]. The design of nanoparticles can be customized for a special application through modulating the properties of nanoparticles such as size, shape and charge[120]. According to the permeability and retention effect of nanoparticles, early researchers payed much attention to nanoparticle delivery on tumors, which could be further reinforced by conjugating tumor-targeting antibodies to the nanoparticles[120-122]. Nowadays these delivery methods are still commonly adopted in clinical application, and many research groups also put the natural bio-distribution of nanoparticle into use for cancer immunotherapy[123].

The release of the drug can be triggered from different nanocarriers *via* the cavitation effects or radiation forces induced by US waves[124]. Nanoparticles have played an important role in ultrasound-mediated drug and gene delivery[125,126]. Compared with microbubbles, the major advantage of nanoparticles is that they can be made small enough to extravasate effectively from the leaky vasculature of some tumors, and the main disadvantages are their complex preparation, instability and toxicity[127]. **Figure 5** shows the schematic of targeted gold nanoparticle (GNP) drug releasing and the enhancement of delivery through the BBB in US irradiation[128].

Many experimental results show that targeted US microbubbles can greatly improve the accuracy of tumor diagnosis, having the advantages of safety, efficiency, good targeting, and strong controllability in the treatment of tumors[129]. Although the application prospect of targeted microbubbles is exciting, there are still several points worth paying attention to: (1) Strengthening the binding ability between microbubbles and ligands, and reinforcing the binding strength between ligands and receptors are the basis for microbubbles to be targeted[130]. Although the antibiotin-biotin complex is currently the most effective targeted binding system, antibiotin is subject to endogenous biotin competition[131]. The main source is egg white or bacteria and other extrinsic proteins with immunogenicity, which may lead to a rejection reaction in clinical application. In addition, antibiotin is a kind of large molecular cation that is prone to form immune complexes in the renal basement membrane with high anion concentration in the body[132]. Therefore, a more ideal ligand connection method is needed[133]. (2) The construction of targeted microbubbles is

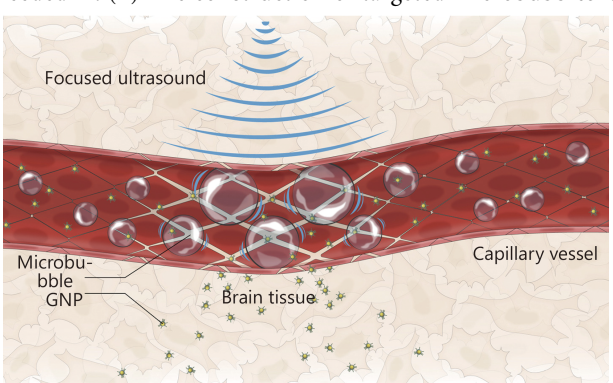

Figure 5 Schematic of the targeted GNP-drug release and the enhanced delivery through the BBB in US irradiation. Adapted from reference 128.

tedious and time-consuming, and their stability and target seeking efficiency *in vivo* needs to be improved[134]. (3) The key technology of targeted microbubbles carrying drugs and genes needs to be improved[135,136]. It is necessary to better protect bioactive substances from shear flow or enzyme degradation during microbubble rupture before entering cells[137]. (4) In order to improve the targeting ability of microbubbles, researches on tumor immunity should be further improved[138]. (5) There are some dangers in the application of ultrasonic microbubbles[139]. The drug particles carried by microbubbles are easily ingested by the liver and spleen[140]. In this case, adverse reactions of different drugs may be relatively large[141,142]. In addition to microbubbles that may cause microembolism and toxicity in blood circulation, the potential risks caused by cavitation nuclei and cavitation effects should not be ignored[143].

The advent of targeted US microbubbles is a revolution in the development of US medicine[144]. With the continuous improvement and optimization of construction technology, and the rapid development of related imaging technology, targeted US microbubbles will be increasingly accelerated into clinical applications, and drugs and gene therapy for tumors will certainly make gratifying progress[145,146].

Low-frequency HIFU enhanced effect of checkpoint inhibitor therapy

Checkpoint blockade antibodies, such as PD-1 and CTLA-4, have demonstrated high efficacy for some extracranial tumors[147-150]. Preclinical and anecdotal clinical evidence showed benefits from treatment of brain malignancies by using checkpoint blockade antibodies[151]. FUS has been used for delivery of antibodies to the brain to treat some diseases, thus, it can be arguably concluded that FUS also has the potential to promote the concentrations of immune-modulating antibodies at the desired site[148,152-154]. Besides the delivery of antibodies, the BBB can be opened with FUS to deliver larger vehicles for drugs and genes, such as liposomes, polymers, polymeric nanoparticles, and virus. The capability of targeted delivery of gene vectors opens up possibilities for altering immune stimuli within the diseased tissue[155].

Checkpoint inhibitor (CI) immunotherapy is playing an important role in the treatment of cancer, but for a subgroup of patients, this treatment is ineffective or even a failure because of drug resistance[156-158]. To improve the CI therapy effect, many concerted efforts are conducted through the use of multiple CIs or use of CIs in combination with other anti-cancer agents. In 2017, the important first report on the efficacy of combining US with CI immunotherapy was published by Silvestrini et al[159]. Their work examined the impact of ablation coupled with aPD-1 and toll-like receptor agonist therapy (CpG). For the case of a single treated tumor, the growth rate of distal tumor is faster using combination therapy than that of aPD-1+CpG only, unless the drugs were administered before ablation. In this paper, an finding was that when two tumors were treated rather than one, abscopal effects were achieved such that the additional non-ablated tumor underwent regression, and survival was improved relative to drug-only treatment. In addition to suggesting the potential of local thermal ablation to affect the treatment of metastatic disease, this highlights the complexity of local treatment on immune effects in that they can be deleterious as well as complementary.

In 2019, Bulner et al.[160] reported the use of "anti-vascular" ultrasound-stimulated microbubble (USMB) treatment in combination with anti-PD-1 CI therapy. Longitudinal growth studies along with acute experiments were conducted by using colorectal cancer cell line CT26 to assess ultrasound-induced anti-tumor immune responses. The results indicated that USMB+anti-PD-1 treatments significantly reinforced tumor growth inhibition and animal survival compared with monotherapies. The ability of anti-vascular USMBs increased the anti-tumor effects of CI therapy, but did not clearly support a T cell-dependent mechanism for the reinforcement.

Conclusion and perspectives

Tumor is a systemic disease. The ideal method of cancer treatment is to remove local tumors without damaging normal tissues, and to activate the whole body's anti-tumor immune response[161]. In recent years, many exciting research results have been achieved in the field of tumor immunotherapy, which bring great hope for the advanced or terminal cancer patient[162]. Due to the high degree of heterogeneity and specificity of the human immune system, current immunotherapy is not perfect yet, and cannot be commonly applied in clinical treatment[163,164].

US has a good prospect in the treatment of diseases, especially in tumor ablation and drug delivery[165]. The use of US alone or the delivery of immune stimulants by US can induce an anti-tumor immune response[166]. The mechanical and cavitation effects produced by FUS can enhance the host's anti-tumor immune response, and deliver genes and antigens to cells to activate the anti-tumor immune response[101]. Compared with HIFU at high temperature aiming for ablating cancer tumor, low-frequency HIFU at low temperature can protect antigen more effectively,

stimulate invasion and maturation of DCs in situ, and trigger stronger immune response[167]. Non-thermal effect of low-frequency HIFU can inhibit the growth and reproduction of cancer cells, damage DNA, and promote apoptosis. After the treatment with low-frequency HIFU, DCs could be recruited to aggregate into the damaged tumor areas, and mainly concentrated in the surrounding of the denatured areas[168]. The combination of low-frequency HIFU and immunotherapy has better results than immunotherapy alone[169].

Many mechanisms of ultrasound-mediated immunotherapy have not been fully understood and need to be further studied[78,170]. First, many factors should be taken into consideration in tumor immunotherapy, such as the patient's cancer type, genetic background, gender, age and so on. According to the action sites, indications and mechanism of different therapeutic drugs, appropriate drug combinations can be reasonably designed in the treatment of diseases. Secondly, low-frequency HIFU at a lower temperature can induce a stronger immune response[171,172]. However, the high-temperature HIFU is more effective for tumor ablation and curing primary diseases, so the advantages and disadvantages of tumor ablation and immunotherapy should be carefully weighed when using HIFU. In addition, as a carrier of genes or antigens in immunotherapy, microbubbles are also complicated to prepare with full consideration of the half-life and penetration efficiency of carriers[162]. It is undeniable that with the gradual thorough research on the mechanisms of immunotherapy, HIFU ablation, microbubble-mediated drug delivery and other mechanisms, the combined treatment of US and immunotherapy has a very broad prospect.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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