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MINI REVIEW

Using ultrasound and microbubble to enhance the effects of conventional cancer therapies in clinical settings

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Abstract
It has been demonstrated in preclinical research that the administration of microbubbles with ultrasound can augment the proapoptotic sphingolipid pathway and enhance chemotherapy or radiation therapy-induced vascular endothelial disruption resulting in enhanced tumor cell death. Specifically, ultrasound-stimulated microbubbles (USMB) can increase blood vessel permeability facilitating the release of therapeutic substances in the target area. USMB can also serve as a potential radiation enhancing therapy as USMB exposure increases tumor cell death significantly as observed in preclinical models. Clinical studies have found the combination of USMB and these existing cancer therapies to be safe and also to be associated with greater tumor responses. USMB-based treatment can be applicable in a clinical setting using either ultrasound imaging or magnetic resonance imaging (MRI) guidance for precise treatment. In the latter, the ultrasound device is integrated into the MRI system platform for sonication to facilitate microbubble stimulation. In this review, we concisely present findings related to USMB and existing cancer therapies (chemotherapy and radiation therapy) in clinical trial settings. The possible underlying mechanism involved in USMB-enhanced chemotherapy or radiotherapy enhancement is also discussed. Lastly, the study concludes with some limitations and an examination of the future direction of these combined therapies.

Keywords Cell death · Chemotherapy · Radiation therapy · Sphingolipids · Ultrasound-stimulated microbubbles

1 Background

Cancer is one of the leading causes of death worldwide. This disease initiates when normal cells start multiplying in an abnormal manner forming a "colony" of cancer cells known as a primary tumor. These cancerous cells can spread to another part of the body and form macroscopic deposits also known as secondary tumors or metastases [1–4]. At present, different types of cancer treatments are available based on the type, size, and anatomical site of the tumor. These include chemotherapy, radiation therapy, surgery, embolization therapy, hormone therapy, thermal ablation, immunotherapy, photodynamic therapy, stem cell transplant, and targeted therapy [5–16]. Depending on the cancer type, patients may receive monotherapy or combined therapy during the course of treatment.

The most common used cancer treatments are surgery, chemotherapy, and radiation therapy. However, surgery is often not possible in cases where cancers are not contained in one area. That makes chemotherapy and radiation therapy the most used treatment options for cancer patients. Data from clinical trials suggest that for most cancer patients, treatment with chemotherapy alone or radiation therapy alone is unable to eradicate cancer cells completely [17–19]. Therefore, combinatorial therapy that can enhance the effects of existing conventional cancer therapies is widely used these days in treating cancers [17, 20–24]. There are currently several radiation enhancers or sensitizers used in combination with radiation therapy to enhance tumor response. Significant research has been conducted in recent years using ultrasound-stimulated microbubbles (USMB) to enhance tumor vascular permeability for cellular drug uptake, as well as to enable

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tumor vessel radiosensitization that ultimately results in enhanced anti-tumor effects [25–30]. Microbubbles are a small micron-sized bubble that ranges between the size of 0.5–10 μm in diameter [31, 32]. They are widely used as a contrast agent for diagnosis with ultrasound as well as for therapeutic purposes [33–35]. When exposed to a certain ultrasound pressure, microbubbles start vibrating and oscillating causing contraction, expansion, and fragmentation [36]. Low ultrasound pressure induces stable cavitation causing the bubbles to contract and expand, whereas higher pressure induces inertial cavitation causing the microbubbles to violently burst in tiny bubbles [31, 37]. Bubble cavitation can cause mechanical perturbation of cells inducing the process of sonoporation [38–40]. Sonoporation induced by USMB is a non-invasive method that allows the delivery and transfer of therapeutic substances to a targeted site without involving any surgical procedure [38, 41]. Additionally, ultrasonic microbubble cavitation can induce gene-expression responses to endothelial cell membrane perturbation which can enhance radiosensitivity [42].

Specifically, numerous preclinical studies have shown that USMB causes mechanical perturbation of endothelial cells that can activate the acid sphingomyelinase (ASMase)-ceramide pathway resulting in endothelial apoptosis, vascular collapse, and ultimately tumor cell death [25, 43–45]. Interestingly, the same phenomenon is observed when using single high doses of radiation (≥ 8 Gy per fraction). The accumulation of ceramide within endothelial cells is known to govern the process of vascular disruption. The effects are reported to be synergistically greater when USMB is combined with radiation [45, 46]. An *in vivo* study on the prostate xenograft model indicated significant vascular disruption and more than tenfold higher tumor cell death within 24 h following a combined treatment of USMB and radiation [25]. Another study using a fibrosarcoma xenograft model demonstrated acute as well as longitudinal tumor vascular effects caused by these combined treatments [47]. Acute vascular collapse was found to be the leading effect of longitudinal tumor response. It was demonstrated that lowering the radiation dose to 2 Gy and combining it with USMB were able to produce sufficient ceramide similar to that of single higher doses of 8 Gy resulting in enhanced tumor response [47]. The involvement of ceramide in inducing *in vivo* tumor response when using chemotherapy and USMB has also been reported [48]. However, the therapeutic involvement of ceramide in clinical studies remains mostly uncharacterized.

In the next section, we discuss the clinical outcomes of studies combining USMB and existing cancer therapies (chemotherapy and radiation therapy). We also discuss the pivotal role of sphingolipids in various types of cancer progression and growth.

2 Clinical findings combining existing cancer therapies

The application of USMB with either chemotherapy or radiotherapy has proven to be clinically feasible. Besides improving the treatment efficacy of these cancer therapies, incorporating USMB can help use lower doses of chemotherapy or radiotherapy, which might prevent adverse side effects caused by higher doses. Most clinical studies currently focus on phase I trials, which assess the preliminary safety and feasibility of combined USMB and cancer treatments. However, further research awaits the need to compare the safety, effectiveness, and therapeutic effects of these treatments.

Kotopoulis et al. utilized USMB combined with gemcitabine to treat patients with pancreatic cancer [49]. In this case study, patients were administered gemcitabine first followed by ultrasound scanning (center frequency = 4.0 MHz; MI = 0.4) and microbubble administration. SonoVue® injection (0.5 ml microbubbles followed by saline injection of 5 ml administered every 3.5 min) was carried out intravascularly along with ultrasound scanning performed for 31.5 min. It was observed that out of five patients, two patients demonstrated reduced tumor size compared to original size from 80 ± 5 to 70 ± 5%. The remaining patients also showed diminished tumor growth. Computed tomography (CT) was used to measure tumor size every 8 weeks. The study showed that the combination of ultrasound and microbubbles allowed an increased uptake of chemotherapy cycles by inducing sonoporation. Patients enrolled in the study were able to undertake and tolerate an increased number of treatments from 9 to 16 cycles [49]. Dimcevski et al. carried out a phase I clinical trial using USMB and gemcitabine in patients with inoperable pancreatic cancer [50]. Patients ($n = 10$) first received an intravenous injection of gemcitabine over 30 min. After this, patients received ultrasound treatment with 0.5 ml SonoVue® followed by 5 ml saline every 3.5 min. When compared between the first and last treatment, five patients demonstrated a decrease in tumor size. Additionally, median survival in all patients ($n = 10$) increased to 17.6 months compared to 8.9 months (historical control group) ($p = 0.011$). The findings also indicated that increasing the number of gemcitabine cycles was tolerated by all the patients compared to control groups [50].

The role of USMB in enhancing the effect of chemotherapy has also been studied in gastrointestinal cancers [51]. Wang et al. conducted a clinical study including eleven patients with hepatic metastases from digestive system tumors and one patient with pancreatic carcinoma. Patients received different types of chemotherapy and different ultrasound pressures with mechanical indexes

of 0.4, 0.6, 0.8, and 1.0. Patients received chemotherapy following a treatment of ultrasound and SonoVue® injection. SonoVue® microbubbles mixed in 5 ml saline were injected into the antecubital vein, followed by a flush with 5 ml saline. Patients received USMB treatment within half an hour of chemotherapy. Outcomes suggested no adverse side effects in any patients. Six patients demonstrated stable disease, and one patient demonstrated partial response. The follow-up session indicated no new lesions formation with restricted tumor progression observed in all patients. A mechanical index of up to 1.0 was found to be safe for treating patients with gastrointestinal cancers [51].

The use of USMB combined with chemotherapy has been frequently used for the treatment of breast cancer patients. A study by Zhou et al. reported that USMB combined with chemotherapy can enhance the treatment effects in patients with HER2-negative breast cancer [29]. The study included 26 patients in total. Patients ($n=10$) were first treated with TAC (taxane (docetaxel), anthracycline (epirubicin or doxorubicin liposomes), and cyclophosphamide) followed by ultrasound scanning and SonoVue® injection (5 ml SonoVue® + 5 ml saline (subsequently followed by 2 ml SonoVue® carried every 4 min) that took place for 20 min. The tumor diameter in all 10 patients receiving treatments was seen to reduce significantly. Additionally, when compared to the control group (patients that received the aforementioned neoadjuvant chemotherapy (NAC)) alone ($n=16$), a higher number of patients in the combined USMB and chemotherapy-treated group demonstrated a pathologic complete response. Furthermore, tumor blood perfusion in patients following treatment was significantly higher as compared to pre-treatment [29].

The therapeutic effect of chemotherapy and USMB was explored in one of the studies by Rix et al. The study investigated the tumor morphology differences in preclinical and clinical studies using breast tumors [52]. The effects of a higher mechanical index (0.8) applied to the entire tumor while administrating chemotherapy (carboplatin) for 18 min were analyzed. In this work, an ultrasound transducer of 7 MHz with pulse lengths of 0.008 ms approximately and a duty cycle of 7% were used. Alongside, an intravenous injection of 0.5 ml SonoVue® (six consecutive injections) was given every 3 min. Doppler images for each tumor were acquired for the estimation of tumor vascularization. Both the preclinical and clinical models received similar treatment of USMB and chemotherapy. When compared with the preclinical data that showed a significant reduction in relative blood volume with a high mechanical index, no changes in patient tumor vascularization were observed with similar parameters used. In addition to this, the size of the blood vessels in the human tumor was found to be larger with increased connective tissue as compared to the mice model with lesser connective tissue and smaller microvessels. A decrease in the number of perfused tumor blood vessels was only seen in the preclinical model with no such observation indicated in patient tumors [52]. These divergent findings in preclinical and clinical models point out the urgent need for more studies to be conducted related to understanding the effects of biophysical parameters of USMB and cancer therapies.

Haram et al. studied the safety and feasibility of USMB and chemotherapy in colorectal cancer patients [53]. Patients ($n=17$) with liver metastases from colorectal cancer were included in the study. Two lesions in each patient were selected and randomized either by treatment with USMB (focused ultrasound and microbubbles) and chemotherapy or remained as a control group that was treated with microbubbles and chemotherapy. CT images were used to select and differentiate the lesions (distant from one another) with one side receiving FUS sonication (experimental group) and other remained non-sonicated (control group). The patients in experimental group were first treated with FOLFIRI or FOLFOXIRI followed by ultrasound treatment with the following parameters: frequency 1.67 MHz, mechanical index 0.5, pulse repetition frequency 0.33 Hz, 33 oscillations, duty cycle $0.2-0.4\%$, and a nine-bolus injection of SonoVue® administered at an interval of 3.5 min with treatment lasting for a total of 35 min. The treatment was reported to be safe; however, the data showed variability in the lesion's response making it difficult for researchers to conclude the outcomes of the study. The therapeutic effects were not studied in this clinical trial [53].

Another study by Sonabend et al. studied the safety of USMB in opening the blood–brain barrier (BBB) for increasing the efficacy of chemotherapy in patients with recurrent glioblastoma [54]. Patients ($n=17$, nine male and eight female) were included in the study. Ultrasound microbubbles (Definity® 10 µl/kg) (68 cycles in total) were used for BBB opening. Side effects like encephalopathy (grades 2, 3), peripheral neuropathy (grade 2), headache (grades 1–2), neutropenia, leukopenia, and hypertension were observed in different patients. Furthermore, results from the biopsy and blood samples confirmed paclitaxel concentration (patients $n=7$; biopsy samples $n=81$; sonicated brain $n=41$ (32 used for hemoglobin analysis); non-sonicated brain $n=40$ (28 used for hemoglobin analysis) and carboplatin concentration (patients $n=3$; biopsy samples $n=48$; sonicated brain $n=23$ (22 used for hemoglobin analysis); non-sonicated brain $n=25$ (23 used for hemoglobin analysis) in brain parenchyma to be 3.7 times and 5.9 times higher, respectively in the sonicated brain (obtained 45 min approximately post sonication) as compared to the non-sonicated ones [54].

One of the standardized treatments for breast cancer includes radiation therapy, as it significantly suppresses the growth of tumors; however, the side effects that come due to high radiation doses cannot be overlooked. Studies

have shown that treating breast patients with radiation therapy alone cannot diminish cancer in most cases. It is reported that a high rate of breast cancer treated with radiation therapy showed symptoms and side effects such as pain, skin discoloration, inflammation, bleeding, shoulder mobility issues, brachial plexopathy, neurological issues, and chances of developing a secondary cancer [55]. By combining USMB with radiation, it is possible to limit the usage of high radiation doses, which can prevent several risk factors. In terms of work with radiation and USMB, Dasgupta et al. conducted clinical trials studying the effects of USMB in radiation enhancement in breast cancer patients [56]. Patients ($n=8$) were included in the study. They first received USMB treatment followed by radiation therapy delivered within 1 h. The USMB treatment parameters used in this study were based on previous preclinical findings that included frequency 800 kHz, peak negative pressure 570 kPa, pulse sequence 16-cycle tone burst over 50 ms, a delay time 1950 ms, and insonication time 7500 ms [25] [56]. Results from 3-month follow-up visits indicated seven patients (eight tumors) with complete response. Acute toxicity was observed in some patients induced upon radiation treatment with dermatitis grades 1 and 2 — the same as expected with radiation alone. No patients were reported to have long-term radiation side effects or systemic reactions due to USMB [56]. Another study by Palhares et al. reported similar effects using 18 patients (20 tumors) with breast cancer [57]. Patients were treated with USMB before radiotherapy. The USMB parameters used in this study was similar to the previous one [56]. Follow-up upon 3 months indicated complete response for 50% of patients, partial response for 33% of patients, stable disease for 11% of patients, and progressive disease for 6% of patients. Subsequent follow-ups of these patients revealed tumor progression at 15 and 8 months for those who showed stable disease ($n=1$) and partial response ($n=1$), respectively. It was reported that no patients had to undergo surgical resection. Treatment side effect seen in some patients was radiation dermatitis including 75% with grade 1, 5% with grade 2, and 10% with grade 3. Systemic complications related to USMB were not reported in any patients [57].

The safety of USMB and radiotherapy has also been confirmed in head and neck cancer patients [58]. A total of 11 patients were included in a study conducted by Palhares et al. for treatment safety analysis purposes. Patients received USMB treatment followed by radiotherapy. Three months follow-up showed acute adverse effects in patients that were reported to be due to radiation treatment indicating six patients with grade 3 toxicity and two patients with grade 2 toxicity. Dermatitis was reported in seven patients with grade $1=2$, grade $2=2$, grade $3=3$, mucositis in eight patients with grade $1=5$, grade $2=2$, grade $3=1$) and dysphagia in nine patients with grade $1=2$, grade $2=1$, and grade $3=6$ with inclusion of five patients who already had baseline dysphagia with grades 2–3. No adverse side effects were reported due to USMB treatment [58].

Recent work by Chen et al. explored the safety and effectiveness of USMB and radiation therapy for opening the BBB to treat patients with glioblastoma multiforme (GBM) [59]. Prior to enrolling onto the clinical trails, patients ($n=6$) received a combination of temozolomide (TMZ), concomitant chemoradiotherapy (CCRT) and bevacizumab (BEV). After that, patients underwent repeated USMB and radiation treatment for a total of 24 sessions. The opening of BBB was carried out within 2 h prior to radiation. The study's outcome revealed three patients with progressive disease (mean 33 days), three patients with stable disease (mean 323 days), and one patient with partial response. Grade 3 necrosis related to re-irradiation was reported in one patient; however, no adverse effects associated with USMB were reported. Alongside the clinical study, a preclinical analysis was carried out that demonstrated an enhanced survival rate of the xenograft model following treatment of USMB and radiation therapy as compared to the radiation-only group [59]. The increase in the therapeutic efficacy of combined USMB and radiation therapy in the preclinical model might hint at a possibility of increased patient survival rate in the future once the USMB and radiation parameters are optimized.

The safety accessibility of USMB in combination with transarterial radioembolization (TARE), which is an internal radiotherapy, was tested in hepatocellular carcinoma patients. In this study by Eisenbrey et al., patients ($n=28$) were first treated with TARE followed by USMB administration either at 1–4 h or 1 and 2 weeks approximately. Improved tumor response was seen in patients receiving the combined treatment of USMB and TARE [60].

A table summarizing the details and goals of the studies is presented in Table 1.

3 Targeting signaling pathway in cancer therapy

Despite technological advancement in cancer treatments, the underlying mechanism that governs cancer growth and its resistance to treatments remains a leading cause of treatment failure [61–63]. Multiple efforts are underway to understand the possible signaling pathways that could be involved in response prediction. One of the most studied signaling pathways in this area of cancer progression and resistance is the sphingolipid-ASMase-ceramide pathway [64–70]. ASMase is a lysosomal enzyme that hydrolyzes sphingomyelin into ceramide and phosphorylcholine [71–74]. Endothelial cells exhibit 20-fold higher secretory ASMase than other cells

Table 1 Details and goals of the studies

Cancer Types	Conventional Cancer Therapies	Microbubbles Types	Studies Purpose/Outcomes	References
Pancreatic cancer	Chemotherapy (gemcitabine)	SonoVue®	Efficacy	[49]
Pancreatic cancer	Chemotherapy (gemcitabine)	SonoVue®	Safety, efficacy and toxicity	[50]
Malignant tumors in the digestive system	Chemotherapy (multiple)	SonoVue®	Safety	[51]
Breast cancer	Chemotherapy (combination of paclitaxel and carboplatin)	SonoVue®	Influence on tumor perfusion	[52]
Breast cancer	Chemotherapy TAC (taxane—(docetaxel), anthracycline—(epirubicin or doxorubicin liposomes), and cyclophosphamide)	SonoVue®	Efficacy and toxicity	[29]
Colorectal cancer (liver metastases)	Chemotherapy (FOLFIRI or FOLFOXIRI)	SonoVue®	Safety and feasibility	[53]
Glioblastoma	Chemotherapy (paclitaxel and carboplatin)	Definity®	Safety and pharmacokinetics analysis of drug concentration	[54]
Breast cancer	Radiation therapy (20 Gy in 5 fractions (3 tumor sites), 30 Gy in 5 fractions (3 tumor sites), 30 Gy in 10 fractions (1 tumor site), and 40 Gy in 10 fractions (2 tumor sites))	Definity®	Safety and response rates	[56]
Breast cancer	Radiation therapy (20 Gy/5 fractions (40%, $n=8/20$), 30 to 35 Gy/5 fractions (35%, $n=7/20$), 30 to 40 Gy/10 fractions (15%, $n=3/20$), and 66 Gy/33 fractions (10%, $n=2/20$))	Definity®	Safety and efficacy	[57]
Head and neck cancer	Radiation therapy (5–10 radiation fractions; 33–35 radiation fractions)	Definity®	Safety	[58]
Malignant high-grade glioma	Radiation therapy (five consecutive days within 1 week, and a full course was 2 weeks (one fraction of 3–4 Gy per day; total dose: 30–40 Gy), including cRT treatment 1 to cRT treatment 10 (cRT 1–cRT 10)) (cRT, conventional radiotherapy)	SonoVue®	Safety	[59]
Hepatocellular carcinoma	Transarterial radioembolization (TARE)	Optison	Safety and feasibility	[60]

[75–77]. Upon various stress stimuli including radiation, and chemotherapeutic drugs, ASMase is known to translocate from lysosome to plasma membrane, once translocated it results in ceramide release. The ceramides first formed are small molecules that self-associate to form a ceramide-enrich membrane domain which then acts as a secondary signaling messenger that emits cell death signal inside the cells [78–84–8683]. In contrast, sphingosine-1-phosphate (S1P), a ceramide antagonist formed by de-acylation of ceramide by ceramidase enzyme, promotes cancer cell survival and progression [67, 87–89]. On the one hand, where ceramide causes endothelial apoptosis, S1P maintains endothelial integrity. Thus, the balance of ceramide-S1P rheostat is required for endothelial homeostasis.

The involvement of these lipid metabolites in deciding the fate of cells has been confirmed in various preclinical models [25, 47, 90–92]. However, their clinical relevance is yet to be elucidated more broadly. Few studies have pointed out variations in the levels of sphingolipids and their connection to cancer progression in patients [93, 94]. A study carried out by Nagahashi et al. showed that the level of sphingolipids, including S1P, sphingosine (Sph), sphingomyelin (SM), monohexosylceramide (HexCer), and ceramides, was found to be higher in breast tissue obtained from breast patients as compared to normal breast tissue [95]. Another

study by Tsuchida et al. revealed that tumor tissues of breast patients with lymph node metastasis demonstrated higher S1P levels compared to lymph node-negative breast cancer patients [96]. Furthermore, Moro et al. demonstrated that breast cancer patients with a high level of ceramide showed less aggressive behaviors as opposed to patients expressing other sphingolipid enzymes demonstrating poor prognosis [97]. These studies confirmed the correlation between sphingolipid molecules and their implication in cancer development. The upregulation of these sphingolipids has also been confirmed in hepatocellular carcinoma patients; however, their link to cancer progression was not determined [98]. Another study by Wątek et al. reported a reduced level of blood ASMase in multiple myeloma patients compared to the control group [99]. All these studies indicate that the level of sphingolipid molecules may increase or decrease in cancer patients depending on the type of cancer.

The modulation of ceramide and its metabolites during the course of cancer treatments has been marked in different cancer patients. Treatment such as hypofractionated stereotactic body radiation therapy (SBRT) has been demonstrated to increase the level of ceramide in cancer patients making it easier for predicting radiosensitivity in patients [100, 101]. A phase II trial conducted by Dubois et al. assessed the involvement of ceramide as a prognostic marker for liver and lung oligometastases of colorectal cancer. Plasma ceramide levels were measured for patients who underwent a treatment of hypofractionated SBRT in combination with chemotherapy (irinotecan). On days 3 and 10 following treatments, the total plasma ceramide levels were reported to be significantly higher than the basal ceramide level (before treatment) in patients who were complete responders ($n=10$) and partial responders ($n=8$). In contrast, the level remained unaltered or reduced significantly in patients with stable disease ($n=8$) and progressive disease ($n=9$) [101]. An earlier study by Sathishkumar et al. explored the role of the ASMase-ceramide pathway in predicting SBRT response in cancer patients [100]. Bulky tumors (n=11) were treated with 15 Gy followed by consecutive multiple doses of 2 Gy. Following treatment, the level of serum ceramide and secretory SMase (S-SMase) was quantified at 24, 48, and 72 h. An elevation in serum S-SMase and ceramide levels was seen in patients who demonstrated partial or complete response at 72 h post-irradiation. In contrast, nonresponder patients depicted no change or increase in S-SMase and ceramide levels. Furthermore, human microvascular endothelial cells (HME-1) were isolated from the patient's serum at 72 h and compared with the control volunteer group. These cells were treated with TNFα (25 ng/ml) or irradiated with 5 Gy (5–10 min after low-density lipoprotein (LDL) addition) which resulted in increased apoptosis by 25% compared to healthy volunteer [100]. This study was the first to report the involvement of the ASMase-ceramide pathway in endothelial cell death in clinical settings.

The outcome of all these aforementioned studies is encouraging; however, no study to date has been conducted describing the synergy between USMB and existing cancer therapies. Based on the preclinical findings, ASMase/ceramide pathway might be the possible underlying mechanism for improving the therapeutic effect of USMB and cancer therapies (Fig. 1).

4 Conclusion, challenges, and future perspectives

In conclusion, USMB can be considered an innovative promising approach that can be used to enhance the effects of existing cancer therapies. The treatment is found to be safe, feasible, and well-tolerable by most patients. However, existing studies are limited to certain types of cancer and have several other limitations, including small sample sizes, variability in types and doses of chemotherapy, differences in dose and fractionation schedules of radiation therapy, and variable patient follow-up. More studies should be conducted involving multicenter trials as it may provide an

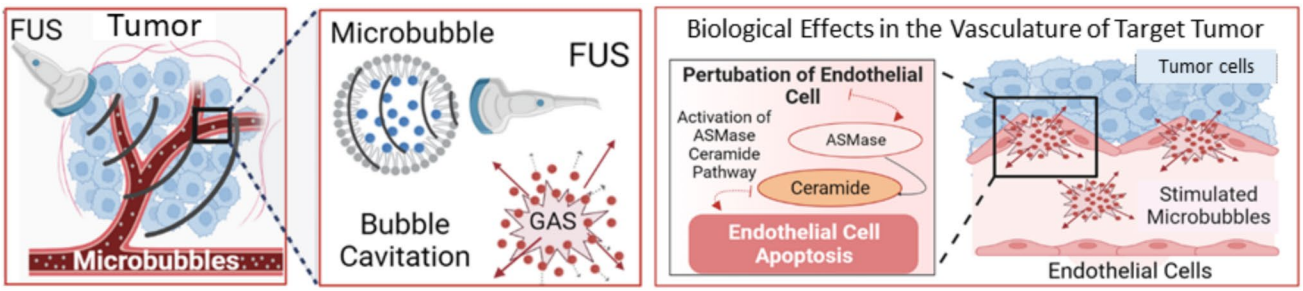

Fig. 1 Underlying mechanism of focused ultrasound (FUS)–stimulated microbubble treatment. Ultrasound-induced bubble cavitation causes activation of the acid sphingomyelinase (ASMase)-ceramide pathway, resulting in endothelial cell death. Enhanced treatment efficacy is achieved by combining ultrasound-microbubble treatment with existing cancer therapies. Adapted from [57]. ASMase, acid sphingomyelinase; FUS, focused ultrasound

advantage for recruiting patients much more quickly as compared to single-centered trials. Also, multicenter trials may allow for diversity in patient coverage and may help with data generalizability. Clinical studies focused on understanding the molecular mechanism in cancer behavior, and its development might be an important reference for clinicians in selecting treatment options. Additionally, studies related to tumor perfusion and activation/involvement of signaling molecules/receptors may be helpful. In particular, the role of sphingolipid metabolism in cancer progression and treatment resistance should be studied in depth. Another valuable study would be to assess if switching the sequence between USMB and existing cancer therapies would lead to different outcomes. Most of the published data incorporating radiation therapy shows that patients are treated with USMB first followed by irradiation. Reversing the treatment regimen and studying its impact on tumor response would be interesting. Few studies have mentioned the difficulty of patient positioning during USMB treatment. A more well-equipped ultrasound system should be designed for the comfort of patients, as bigger tumors might take longer to be treated and patients may have to remain immobile during treatment. Also, the present ultrasound system used in clinical settings operates on a step-by-step approach to activate each treatment cell. This remains a tedious approach as it takes longer time for treatment. To shorten the treatment durations, the next generation of ultrasound therapy system should include automatic simultaneous activation of all treatment cells.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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