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REVIEW

Advances in Ultrasound-Targeted Microbubble Destruction (UTMD) for Breast Cancer Therapy

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Abstract: Breast cancer is one of the most common types of cancer in women worldwide and is a leading cause of cancer deaths among women. As a result, various treatments have been developed to combat this disease. Breast cancer treatment varies based on its stage and type of pathology. Among the therapeutic options, ultrasound has been employed to assist in the treatment of breast cancer, including radiation therapy, chemotherapy, targeted immunotherapy, hormonal therapy, and, more recently, radiofrequency ablation for early-stage and inoperable patients. One notable advancement is ultrasound-targeted microbubble destruction (UTMD), which is gradually becoming a highly effective and non-invasive anti-tumor modality. This technique can enhance chemical, genetic, immune, and anti-vascular therapies through its physical and biological effects. Specifically, UTMD improves drug transfer efficiency and destroys tumor neovascularization while reducing toxic side effects on the body during tumor treatment. Given these developments, the application of ultrasound-assisted therapy to breast cancer has gained significant attention from research scholars. In this review, we will discuss the development of various therapeutic modalities for breast cancer and, importantly, highlight the application of ultrasound microbubble-targeted disruption techniques in breast cancer treatment.

Keywords: ultrasound-targeted microbubble destruction, breast cancer, microbubble, chemotherapy, TME, radiofrequency ablation

Introduction

Breast cancer has emerged as the most prevalent cancer among women globally, accounting for 11.7% of all cancer cases.[1] Alarmingly, projections indicate that by 2040, the global incidence of breast cancer could increase by a staggering 40%.[2,3] Despite significant advancements in medical care that have improved survival rates, the challenges remain substantial. Approximately 20–30% of patients still face the risk of recurrence, leading to poor prognoses and other burdens. Consequently, breast cancer remains the leading cause of death among middle-aged women worldwide.[4,5] In clinical practice, the specific subtype of breast cancer plays a crucial role in determining treatment options and predicting patient prognosis.[6] Breast cancer is categorized based on the detection and analysis of certain molecular markers in patients. These markers help divide breast cancer into four main subtypes: Luminal A, Luminal B, HER2-positive, and triple-negative[7] (Figure 1). Individualized treatment for Luminal and HER2-positive breast cancer typically involves a combination of surgical procedures, chemotherapy, endocrine therapy, and targeted therapy. Advanced stages of the disease are associated with higher malignancy, earlier recurrence and metastasis, and poorer prognoses, which underscores the importance of early and aggressive intervention.[8–10] Triple-negative breast cancer (TNBC) is distinguished by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression, making it the most aggressive subtype of invasive breast cancer. Characterized by rapid progression and a pronounced tendency for recurrence and metastasis, TNBC poses significant clinical challenges. Patients with TNBC typically do not benefit from endocrine therapy or HER2-targeted therapies due to the lack of hormone receptor and HER2 gene expression. This lack of therapeutic targets has historically rendered TNBC one of the most difficult to treat.[11,12] In cases of highly malignant breast cancers, including TNBC, clinicians often employ neoadjuvant systemic therapies to enhance patient survival and quality of life, and potentially achieve a cure. These therapies typically include

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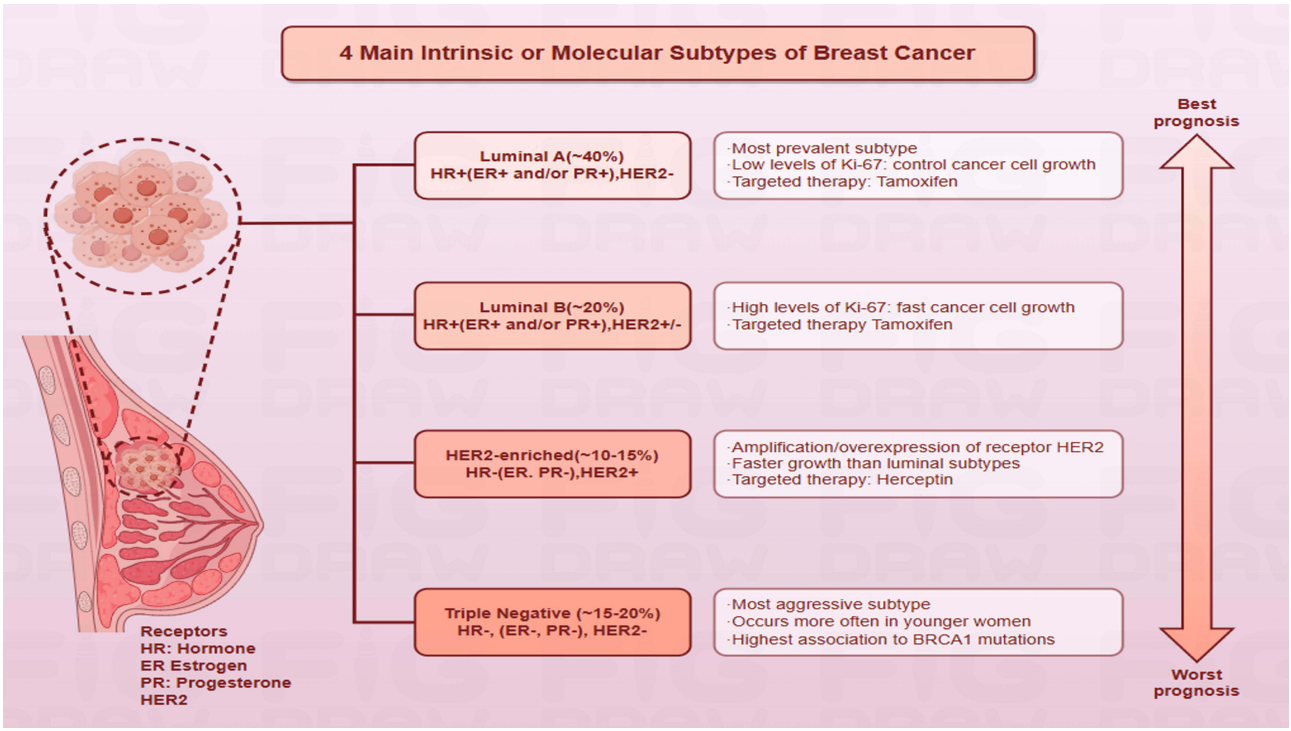

Figure 1 Breast cancer subtypes, their origin and staging.

cytotoxic chemotherapy, hormone therapy (where applicable), and targeted therapies directed at specific tumor cell characteristics.[13] However, recent advancements in immune checkpoint blockade (ICB) offer a novel approach to targeted therapies. Unlike conventional therapies that target tumor cells directly, ICB modulates the tumor microenvironment (TME), thereby influencing tumor growth even in the absence of readily targetable molecular alterations within the tumor cells. To further advance precision oncology in TNBC, a more comprehensive molecular characterization of TNBC subtypes is essential. This molecular typing is crucial for guiding the development and implementation of personalized therapies. The emerging use of immune checkpoint inhibitors (ICIs) and antibody-drug conjugates (ADCs) exemplifies the promise of these new therapeutic strategies, offering hope for improved outcomes in this challenging breast cancer subtype.[14,15] For instance, drugs targeting CDK4/6i have significantly inhibited tumor growth in patients with HR+/HER2- breast cancer.[16,17] However, despite the development of numerous anti-cancer drugs and therapies, most treatments are limited by drug resistance or relapse, rendering them ineffective.[18,19] The underlying mechanism for these therapeutic challenges lies in the tumor microenvironment, which offers a sanctuary and optimal conditions for tumor cell survival and growth. Moreover, it 'shields' and 'promotes' their malignant biological behaviors.[20–22] Consequently, researchers have shifted their focus towards understanding how drugs can penetrate the tumor microenvironment to effectively target and eliminate tumor cells and enhance anti-tumor immunity.

Ultrasound-targeted microbubble destruction (UTMD), an emerging non-invasive therapeutic modality, has demonstrated enhanced drug transfer efficiency and tumor neovascularization disruption via its multifaceted physicobiological effects. Consequently, UTMD exerts synergistic effects in tumor-related therapies, encompassing chemical, genetic, immune, and anti-vascular modalities.[23–25] Furthermore, UTMD can be integrated with acoustic power therapy and composite nanoparticles to potentiate anti-tumor efficacy, thereby offering a novel avenue for targeted tumor therapy. In a seminal study, the intra-tumoral delivery of STAT3 transcription factor decoys into squamous cell carcinoma (SCC) tumors using UTMD resulted in the significant abrogation of STAT3 signaling, leading to markedly suppressed tumor growth.[26] Similarly, in another pivotal investigation, UTMD-mediated delivery of siRNA-loaded nanobubbles (siRNA-NBs) targeting PDLIM5 in human non-small cell lung cancer PC9GR cells effectively silenced PDLIM5 expression, induced autophagy, and promoted both growth inhibition and apoptosis.[27] These findings collectively underscore the

potential of UTMD as a versatile and effective tool in the armamentarium of anti-cancer therapeutics.[27] Moreover, UTMD technology has also been explored in the context of breast cancer therapy, wherein it was utilized to induce reactive oxygen species (ROS) production, thereby modulating the miR-200c/ZEB1 axis and suppressing the epithelial-to-mesenchymal transition (EMT) properties of breast cancer MDA231 cells, as well as inhibiting the migration of breast cancer tumor cells.[28] Furthermore, a recent study demonstrated the efficacy of UTMD technology in delivering dual-targeted cationic microbubbles, which augmented gene transfection efficiency, enhanced ultrasound molecular imaging of tumors, and exhibited pronounced tumor growth inhibition in in vivo experiments, with favorable safety and efficacy profiles.[29] Collectively, these findings highlight the potential of UTMD technology to surmount the limitations of emerging breast cancer treatments, rendering it a promising adjunctive therapeutic modality for the management of this disease.[30]

This review commences with an overview of the various subtypes of breast cancer, elucidating the distinct treatment modalities and therapeutic challenges associated with each. We specifically delve into the tumor microenvironmental factors contributing to drug resistance and suboptimal treatment efficacy, as elucidated by recent advancements in ultrasound oncology. Subsequently, we summarize the clinical status and potential of ultrasound-targeted microbubble destruction (UTMD) in combination with emerging therapeutic modalities for breast cancer patients.

Mechanisms of Ultrasound Therapy in Tumor Treatment

Ultrasound-Targeted Microbubble Destruction (UTMD), an innovative non-invasive precision oncology technique, has garnered considerable attention in tumor and tumor microenvironment therapy over recent years.[23] This technology capitalizes on the synergy between ultrasound and microbubbles to facilitate targeted drug, gene, and other therapeutic agent delivery, followed by site-specific release. Consequently, UTMD enhances therapeutic efficacy while minimizing collateral damage to healthy tissues[24,31](Figure 2).

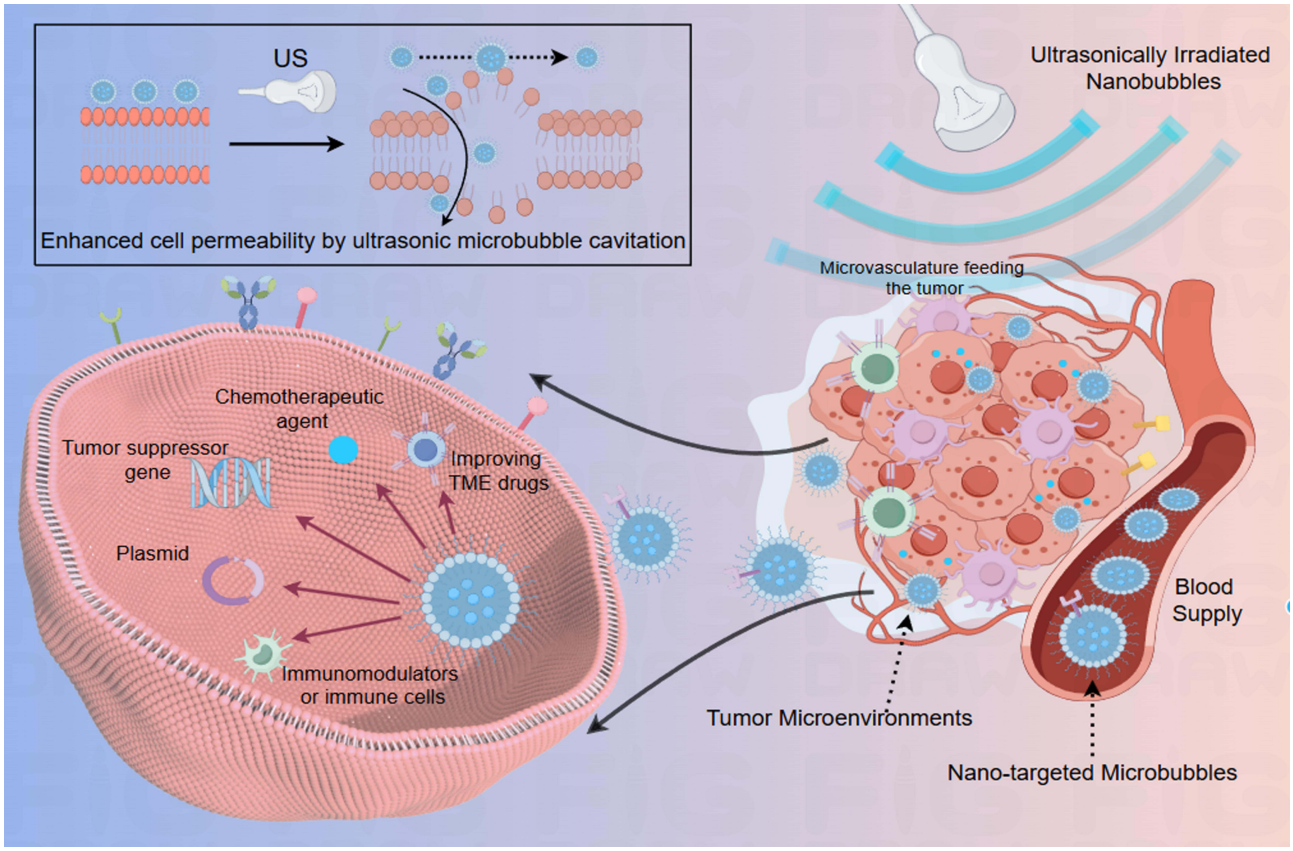

Figure 2 UTMD combined with nano microbubble therapy for tumor treatment.

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The application of ultrasound in tumor treatment primarily encompasses high-intensity ultrasound and low-frequency, low-intensity ultrasound. High-intensity ultrasound principally eliminates or damages tumor cells directly through thermal and mechanical effects (eg, High Intensity Focused Ultrasound, HIFU).[32–34] Clinically, HIFU focuses ultrasound waves to generate localized high temperatures, thereby destroying tumor tissues.[35,36] In contrast, low-frequency, low-intensity therapeutic ultrasound, with frequencies ranging from 20 kHz to 1 MHz and intensities between 0.1 to 3.0 W/cm², is characterized by its deep tissue penetration, low tissue attenuation, and minimal damage to normal cells. This modality can enhance the efficacy of antitumor drugs such as curcumin in glioma cells when combined with microbubbles.[37,38] Moreover, the targeted and non-invasive nature of low-frequency ultrasound allows it to act as a carrier for gene and drug delivery, inducing apoptosis in tumor cells and blocking tumor microvessels.[39,40] In recent years, Ultrasound-Targeted Microbubble Destruction (UTMD) has emerged as a non-invasive treatment method that leverages low-frequency ultrasound and microbubbles to enhance the therapeutic efficacy on tumors by improving drug or gene delivery efficiency.[41] The effects of UTMD are multifaceted, encompassing enhanced drug permeability, improved tumor microenvironment, and stimulated immune responses, among other benefits.[42,43] Notably, studies have demonstrated that the combination of low-frequency ultrasound and microbubble cavitation can induce apoptosis in various tumor cells, including those associated with breast cancer, bladder cancer, and prostate cancer, ultimately facilitating targeted tumor cell destruction through increased sensitivity to chemotherapeutic agents.[44,45] Further research has explored the application of UTMD in miR-34a-mimic delivery to tumor tissues. An in vitro cellular assay revealed that upon localized irradiation of the tumor surface following microbubble injection, ultrasound-triggered cavitation at the target site effectively inhibited tumor growth.[46,47] These findings underscore the potential of UTMD in localizing gene or drug delivery to tumors, thereby improving treatment efficacy. Additionally, animal model studies have demonstrated the efficacy of UTMD in enhancing the delivery of therapeutic agents into the interior of solid tumors, thereby reducing the required dose of chemotherapy without compromising treatment efficacy.[48,49] This significant reduction in dosage can lead to diminished side effects, making UTMD a promising approach in the treatment of various cancers. Previous research has investigated the silencing of breast cancer-associated genes, such as MTDH, using UTMD technology. The findings of this study indicate that UTMD is more efficacious than liposome transfection alone in reducing MTDH expression levels and consequently inhibiting tumor growth.[23] UTMD technology represents an innovative and effective strategy for cancer treatment.[50] It not only enhances the accumulation of chemotherapeutic agents or gene therapies within tumor tissues but also minimizes damage to normal tissues, thus exhibiting considerable potential for clinical translation.

Within the tumor microenvironment, the rapid proliferation of tumor cells outpaces the development of an adequate neovascular system, leading to the formation of hypoxic regions. This hypoxic microenvironment not only facilitates the maintenance and proliferation of tumor cells but is also intrinsically linked to enhanced tumor aggressiveness and resistance to chemotherapy and radiotherapy.[51,52] To address the challenge of oxygen deprivation, researchers have employed various strategies, such as the development of oxygen-sufficient nanobubbles. These nanobubbles release oxygen at the tumor site upon activation by ultrasound, thereby increasing local oxygen concentration.[53] Moreover, the application of UTMD technology has been demonstrated to modulate the tumor microenvironment, including the disruption of tumor tissue barriers and the promotion of drug penetration. For instance, a study employed a paclitaxel prodrug that was activated under hypoxic conditions and combined it with a photosensitizer to form nanoparticles. These nanoparticles exhibited enhanced cytotoxicity towards cancer cells upon exposure to light, highlighting the potential of UTMD-based approaches in cancer therapy.[54] Another investigation demonstrated the potential of UTMD in facilitating the delivery of IR780 and oxygen-enriched nanoparticles to tumor sites, thereby promoting the generation of reactive oxygen species (ROS) and inducing apoptosis in cancer cells.[53] These findings collectively suggest that UTMD represents a promising strategy to overcome the limitations of tumor resistance and sensitivity. The UTMD approach not only enhances the tumor microenvironment by promoting drug penetration and delivery to tumor cells, but also potentially reduces the systemic toxicity associated with tumor treatment by allowing for targeted drug release.[55] Moreover, UTMD may modulate vascular structure and boost the immune response to augment the overall therapeutic effect. However, as an emerging field, further clinical trials are necessary to validate the safety and efficacy of UTMD technology.

Therapeutic Strategies and Challenges Associated with Various Pathological Subtypes of Breast Cancer

Breast cancer, a heterogeneous and complex disease, is driven by a confluence of genetic, hormonal, and environmental factors.[56,57] A critical mediator in breast carcinogenesis and progression is the Wnt/β-catenin signaling pathway.[58] Clinically, breast cancer subtypes, including estrogen receptor-positive (ER+), progesterone receptor-positive (PR+), human epidermal growth factor receptor 2 overexpressing (HER2+), and triple-negative breast cancer (TNBC), exhibit distinct therapeutic responses.[10,59] In HER2-positive breast cancer, targeted therapy with anti-HER2 agents such as trastuzumab (Herceptin), often in combination with chemotherapy, has demonstrated significant clinical benefits.[60,61] Conversely, endocrine therapy remains the mainstay for ER+ or PR+ breast cancer.[62] The presence of estrogen receptor (ER) and/or progesterone receptor (PR) in hormone receptor-positive (HR+) breast cancer defines a clinically significant subgroup highly responsive to endocrine therapy. This treatment modality leverages the dependence of these tumors on hormonal stimulation for growth, employing agents like tamoxifen and aromatase inhibitors to effectively block estrogen's actions.[63,64] In sharp contrast, triple-negative breast cancer (TNBC) is defined by the absence of ER, PR, and HER2, rendering endocrine and anti-HER2 targeted therapies ineffective. The absence of these established therapeutic targets necessitates the ongoing development of innovative approaches, such as immunotherapy and targeted therapies directed against alternative pathways critical for TNBC pathogenesis and progression.[65,66] This active area of investigation holds considerable promise for improving outcomes for TNBC patients.[67,68] Beyond the common subtypes, special types of breast cancer, such as invasive lobular carcinoma (ILC), have distinct characteristics that necessitate tailored treatment approaches. ILC, for instance, responds poorly to neoadjuvant chemotherapy, highlighting the need for more individualized treatment regimens.[69,70] The advent of immunotherapy has recently brought renewed hope to the management of these and other breast cancer subtypes. However, the limitations of existing treatments should not be overlooked. Surgical treatment, while essential, faces challenges in completely eradicating metastatic tumor tissue. Chemotherapy, though systemic, is hampered by low selectivity, significant toxicity, and limited patient tolerability. Radiotherapy, while effective, can induce side effects such as radiation dermatitis and myelosuppression. Immunotherapy, while promising, can result in skin, gastrointestinal, and hepatic toxicities.[71,72] Moreover, the tumor microenvironment (TME) plays a crucial role in cancer progression, with immunosuppressive cells potentially impeding the function and persistence of chimeric antigen receptor T-cell (CAR-T) therapies.[73,74]

Additionally, the inherent heterogeneity of breast cancer poses significant challenges to treatment. This diversity is reflected in variations in histological features, molecular profiles, and clinical behaviors, ultimately contributing to the complexity of therapeutic decision-making. Ongoing research aims to elucidate the underlying mechanisms and develop more targeted and personalized treatment strategies to overcome these hurdles.

Ultrasound-Targeted Microbubble Destruction Technology in Adjuvant Breast Cancer Therapy

UTMD in the Chemotherapy of Breast Cancer

Breast cancer chemotherapy plays a pivotal role in the treatment of breast cancer (Table 1). While significant progress has been made in extending patient survival and reducing tumor recurrence, several challenges persist. Notably, conventional chemotherapy methods often face issues with non-specificity and high toxicity.[75] To address these challenges, the integration of nanocarriers or other drug delivery systems can enhance drug specificity and mitigate toxic side effects.[76,77] In a recent study, researchers developed lipid microbubbles loaded with paclitaxel (PTX) and LyP-1 peptides to validate their in vitro tumor targeting efficiency and chemotherapeutic efficacy. The results demonstrated that the targeted drug-loaded microbubbles exhibited efficient and stable attachment to breast cancer cells under both static and dynamic conditions. Furthermore, PTX-loaded microbubbles (MBs) significantly enhanced the anti-tumor effects of chemotherapy. This study highlights the potential of targeted drug delivery systems in improving the specificity and effectiveness of chemotherapy, thus offering a promising avenue for future breast cancer treatments.[78] The results indicated that the targeted drug-loaded microbubbles effectively and stably attached to breast cancer cells under both static and dynamic conditions. Moreover, paclitaxel (PTX)-loaded microbubbles (MBs) significantly enhanced the anti-

Table 1 | Studies on the Use of UTMB in the Treatment of Breast Cancer

Tumor Treatment Modality	Applied Microbubble Classes	Tumor cell type	UTMD Combined Therapy Killing Machine	Tumor Treatment Results
Targeted Cationic Microbubbles Conjugated with CD105 Antibody[83]	CMB105	MDA-MB-231	Microbubbles, loaded with the endothelial marker CD105, increase local gene concentration and mediate targeted aggregation, resulting in significant tumor cell apoptosis[84]	Angiogenesis and tumor cell invasion were successfully inhibited in vitro, with apoptosis and tumor growth inhibition observed in vivo[83]
Enhanced therapeutic effect of Adriamycin on multidrug resistant breast[85]	ABCG2-siRNA	MCF-7	The ABCG2-siRNA-loaded nanoparticles, combined with UTMD, efficiently silenced the ABCG2 gene and enhanced the susceptibility of MCF-7/ADR cells to adriamycin (ADR)[85]	The siRNA-loaded nanoparticles, combined with UTMD and adriamycin (ADR), exhibited a superior tumor inhibition effect and good safety in vivo[85]
Facilitating the accumulation of porphyrin and siRNA at the tumor site through the cavitation effect[86]	SiHIF@CpMB	MDA-MB-231	HIF-1α siRNA down-regulated HIF-1α levels, induced by the common hypoxic tumor environment or ROS generated by photodynamic therapy (PDT). This enhancement of PDT efficacy partly inhibited tumor progression[86]	Utilizing UTMD technology, in situ efficient accumulation of siHIF@CpMBs at tumor sites was achieved, significantly enhancing the efficacy of combined therapy[86]
SiRNA inhibit the proliferation of estrogen-dependent ER+ breast cancer (BC)[87]	CpMBs-PGL-NH2	MCF-7	Amino groups can adsorb siRNA through electrostatic interactions to facilitate FOXA1 knockdown (KD), thereby inhibiting the proliferation of estrogen-dependent ER+ BC[87]	CpMBs/siRNA, combined with ultrasound-targeted microbubble destruction (UTMD), significantly augmented the local accumulation of porphyrin and siRNA via ultrasound-induced sonoporation, as guided by contrast-enhanced ultrasound (CEUS). This approach demonstrated excellent therapeutic efficacy for estrogen-dependent ER+ breast cancer[87]
Paclitaxel (PTX) interfering with the mitotic spindle, resulting in cell cycle arrest and ultimately apoptosis[82]	PTX@RGD-MBs	MDA-MB-231	An effective drug carrier system for precisely delivering PTX into TNBC cells reduces side effects and enhances therapeutic efficacy[82]	In vitro and in vivo ultrasonic experiments demonstrated that PTX@RGD-MBs yielded high-quality contrast-enhanced ultrasound (CEUS) images, thus improving the diagnosis and evaluation of triple-negative breast cancer (TNBC)[82]

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Doxorubicin (DOX) is a first-line chemotherapeutic drug for breast cancer treatment. By enhancing ROS production in cancer cells, DOX induces oxidative stress-mediated cell death[88]	DSPC:DSPE-PEG2000	4T1 cells	UTMD-induced reactive oxygen species (ROS) elevation in tumor regions can enhance the tumor-killing effect of DOX, which is dependent on intratumoral ROS levels. Therefore, the antitumor efficacy of the combination treatment was investigated[88]	UTMD offers a novel, simple, and non-invasive technique for tumor-targeted drug delivery. When combined with chemotherapy, UTMD holds significant potential to enhance the antitumor efficacy of chemotherapeutic drugs[88]
Doxorubicin (Dox) and paclitaxel (PTX), commonly used in breast cancer management, were selected as chemotherapies, with Rose Bengal (RB) serving as the sonodynamic therapy (SDT) sensitizer[89]	PTX+Dox+RB+US	MCF-7	Using MBs to facilitate delivery of chemo-sonodynamic therapy for the treatment of breastcancer[89]	Animals receiving microbubble treatment maintained constant weight throughout the study, whereas those treated with a Cremophor suspension of PTX/Dox exhibited a 12.1% weight reduction[89]
Zwitterion-modified gadolinium (Gd)-chelated core–shell tecto dendrimers (CSTDs) were employed as a nanomedicine platform (PCSTD-Gd) for enhanced magnetic resonance (MR) imaging-guided chemo-gene therapy of orthotopic breast cancer, assisted by UTMD[90]	PCSTD-Gd/DOX/miR 21i	MDA-MB-231	Multifunctional PCSTD-Gd/DOX/miR-21i polyplexes, delivered to tumors with the assistance of UTMD, exhibited enhanced tumor penetration and enrichment for MR imaging and combination treatment of TNBC[90]	PCSTD-Gd/DOX/miR-21i polyplexes, facilitated by UTMD, enabled enhanced in vivo MR imaging-guided chemo-gene therapy of an orthotopic breast cancer model[90]
Simvastatin (SIM), a clinically used lipid-lowering medication, is a well-established inhibitor of the mevalonate (MVA) pathway with additional anticancer effects, as supported by clinical trial results[91]	SIM-NDs	MDA-MB-231	As a key inhibitor of ferroptosis, the selenoenzyme glutathione peroxidase 4 (GPX4) is a classical therapeutic target. UTMD may be employed to enhance the delivery and efficacy of GPX4 inhibitors in ferroptosis-based cancer therapy[91]	The combination of UTMD and Self-Assembled Ionic Micelles of Nanodiscs (SIM-NDs) offers a promising strategy for inducing ferroptosis in the treatment of malignant tumors[91]
PROTACs harness E3-ubiquitin ligases to achieve efficient protein degradation, thereby mitigating drug resistance due to target overexpression or mutation. This approach offers several advantages, including repeatability, low administration doses, and the ability to degrade previously undruggable proteins[92]	ARV-MBs,	MDA-MB-231	ARV-MBs, in combination with ultrasound irradiation, enhanced PROTAC delivery and permeability, resulting in significant in vitro and in vivo cancer toxicity[92]	Under ultrasound, ARV-MBs mediated BRD4 ubiquitination and degradation, resulting in an effective antitumor effect[92]

(*Continued*)

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Table 1 (Continued).

Tumor Treatment Modality	Applied Microbubble Classes	Tumor cell type	UTMD Combined Therapy Killing Machine	Tumor Treatment Results
SOCS3 overexpression inhibits the activity of JAK/STAT3 signaling pathway in breast cancer cells, inhibits cell proliferation, and improves the sensitivity to ADM-induced apoptosis[93]	UTMD- and liposome-mediated SOCS3	MCF-7	UTMD and liposome-mediated SOCS3 reduced cell viability, proliferation, migration and invasion, blocked cell cycle, inhibited sphere formation in BCSCs, and retarded tumor growth in mice[93]	UTMD increased the transfection rate of SOCS3. Furthermore, UTMD and liposome-mediated SOCS3 reduced cell viability, proliferation, migration, and invasion, blocked cell cycle progression, inhibited sphere formation in BCSCs, and retarded tumor growth in mice[93]
Inhibition of MTDH expression by shRNA interference effectively reduces breast cancer metastasis[23]	MTDH by shRNA using liposome	MCF-7, MCF-10A, and T47D	UTMD plus liposomes worked well together to deliver shRNA efficiently, reducing the expression of MTDH. This, in turn, decreased the ability of MCF-7 cells to survive, move, invade, and undergo EMT[23]	Liposomal-UTMD delivery enhanced shRNA transfection, suppressing MTDH and thereby inhibiting MCF-7 cell proliferation, migration, and EMT[23]
MiR-145-5p inhibits BC cell growth, migration and invasion by negatively regulating ACTG1 levels in BC cells[94]	miR-145-5p-MB	MCF-7 and MDA-MB-231	UTMD improved the therapeutic delivery of miR-145-5p and potentiated its inhibitory effects on BC cell malignancy[94]	UTMD targeted delivery technique, boosted the therapeutic potential of miR-145-5p in BC cells. UTMD may enable localized miRNA therapy[94]

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tumor effects of chemotherapy. Another significant challenge in breast cancer chemotherapy is multidrug resistance (MDR). The development of novel drug delivery systems using ultrasound-targeted microbubble disruption technology can provide precise control over chemotherapeutic drug delivery, thereby improving therapeutic efficacy and reducing adverse effects.[79,80] Studies have shown that innovative drug carrier systems or nanosystems, such as those using modified graphene oxide and doxorubicin, can reverse MDR by inhibiting the drug efflux transport protein P-glycoprotein (P-gp).[81] These advancements underscore the potential of targeted drug delivery systems and nanotechnology in addressing major challenges in breast cancer chemotherapy, thereby enhancing treatment outcomes and reducing side effects. In a recent study, researchers employed paclitaxel-loaded lipid microbubbles (PTX@RGD-MBs) in conjunction with ultrasound-targeted microbubble disruption (UTMD). This approach significantly enhances the diagnostic and therapeutic efficacy of Triple Negative Breast Cancer (TNBC) by leveraging the mechanical effects of ultrasound, such as the vibration and rupture of microbubbles (MB) or nanobubbles (NB). This process increases drug concentration and penetration at the tumor site.[82] In conclusion, the combination of UTMD with a targeted drug delivery system facilitates the localized delivery of drugs to the tumor site through ultrasound-mediated microbubble destruction. This method effectively enhances drug concentration while reducing systemic toxicity, thereby avoiding the serious side effects typically associated with traditional chemotherapy.

UMTD in the Radiotherapy of Breast Cancer

Radiation therapy (RT) plays a pivotal role in breast cancer management, reducing local recurrence following mastectomy or breast-conserving surgery.[95,96] The seminal study at Guy's Hospital in 1960 established the safety and efficacy of post-lumpectomy RT,[97] with subsequent trials confirming its benefit in reducing local recurrence.[98,99] However, the double-edged sword of RT's tumoricidal power and associated toxicities, such as cardiotoxicity, has driven advances in our understanding of breast cancer biology and RT techniques.[100,101] Subsequent advances in breast cancer biology and radiation oncology have enabled the personalization of RT, optimizing patient selection, treatment techniques, and fractionation schedules.[102,103] However, despite these improvements, challenges remain, including radiation-induced skin reactions, cardiac toxicity, and myelosuppression.[104] Ultrasound molecular tomography (UTMD) offers a potential role in enhancing treatment efficacy by targeting tumor microvasculature and blood flow dynamics.[105,106] Several studies have investigated the synergistic effects of radiation therapy and ultrasound-guided focused ultrasound (FUS) in preclinical breast cancer models. These studies employed ultrasound stimulation of microbubbles to induce vascular damage, thereby enhancing tumor radiosensitivity. This approach has demonstrated compromised tumor vasculature and improved response to radiation therapy.[107,108] Furthermore, the application of ultrasound-mediated drug delivery (UTMD) technology offers a promising avenue for targeted therapeutic interventions. For instance, UTMD has successfully facilitated the delivery of microRNAs, such as miR-21-5p inhibitors, into lung cancer cells, and this approach has demonstrated efficacy in preclinical breast cancer models, potentially improving treatment efficacy and mitigating adverse effects.[109] Furthermore, research has demonstrated that ultrasound facilitates the infiltration of nanomaterials into tumor stroma, thereby enhancing the efficacy of in vivo radiotherapy and chemotherapy, and reducing tumor microvascular density and cell proliferation markers.[110] Emerging evidence suggests that the combination of UTMD and radiotherapy holds significant promise for improving treatment outcomes and prognosis in breast cancer.

UTMD in Targeted Immunotherapy for Breast Cancer

Immune checkpoint inhibitors (ICIs) represent the foremost class of therapeutics currently employed in breast cancer immunotherapy. These agents potentiate the immune system's anti-tumor response by inhibiting the PD-1/PD-L1 pathway, thereby relieving the suppression of T cells.[111] A notable example is pembrolizumab (Keytruda), a PD-1 inhibitor that has demonstrated significant efficacy, particularly in the treatment of triple-negative breast cancer (TNBC).[112] Notably, ICIs shift the therapeutic paradigm by explicitly targeting the tumor microenvironment (TME) rather than the tumor cells themselves. This strategy offers a novel approach to targeted therapy, even in cases where the tumor cells lack conventional therapeutic targets.[113,114] However, thanks to immune checkpoint blockade (ICB), explicitly targeting the tumor microenvironment (TME) rather than the tumor cells themselves provides a new approach to targeted therapy, even if the tumor cells lack actionable targets. Components of the TME can contribute to disease

progression by secreting and expressing factors that stimulate tumor cell proliferation and suppress anti-tumor immunity, or conversely, contribute to tumor control through adaptive immune mechanisms.[115] The pivotal role of immunity in cancer underscores the necessity for immunotherapeutic agents to effectively penetrate the TME to reach tumor cells, engage with PD-1, and subsequently modulate the response of immune effector cells, including macrophages, natural killer (NK) cells, and dendritic cells, ultimately enabling effective tumor cell elimination.[116–118] Recent research has demonstrated that ultrasound-targeted microbubble destruction (UTMD) can effectively modulate the tumor immune microenvironment, presenting a promising strategy for tumor immunotherapy.[119,120] The synergistic action of ultrasound and microbubbles enhances the tumor immunosuppressive microenvironment by inducing antigen release from tumor cells, both mechanically and thermally. This process facilitates antigen presentation and promotes T-cell recognition and subsequent killing of tumor cells. Consequently, UTMD may address some of the challenges inherent in traditional therapeutic approaches such as immune checkpoint blockade (ICB) and chimeric antigen receptor (CAR)-T cell therapy.[24,121] Several studies have investigated the efficacy of ultrasound-mediated drug delivery in targeting HER2-positive breast cancer. Elamir et al demonstrated that liposome-encapsulated anti-HER2 monoclonal antibodies, released via microbubble-triggered sonoporation at low frequencies, exhibited superior tumor growth inhibition compared to other treatment modalities. This enhanced efficacy was attributed to increased drug efficiency, reduced cytotoxicity, promotion of apoptosis, and stimulation of an antitumor immune response.[122] These findings corroborate the work of Callmann et al, who also observed that ultrasound stimulation facilitated targeted drug release in HER2-positive breast cancer cells, resulting in improved tumor suppression and decreased systemic toxicity. Further research is needed to elucidate the precise mechanisms underlying these observed benefits and to optimize the parameters of ultrasound-mediated drug delivery for clinical translation.[123] In a recent study, a novel ultrasound-responsive spherical nucleic acid (SNA) system was developed to target c-Myc and PD-L1 in triple-negative breast cancer (TNBC). This self-assembled, vector-free small interfering RNA (siRNA) system selectively inhibits c-Myc and PD-L1 in cancer cells when activated by ultrasound, thereby enhancing therapeutic efficacy against TNBC.[124] Additionally, the combination of ultrasound-stimulated microbubbles and hyperthermia (USMB and HT) has shown promise in targeting breast tumor vasculature, with potential therapeutic benefits demonstrated in preclinical studies.[125] Furthermore, ultrasound-stimulated microbubble cavitation (USMC) technology has been employed to enhance drug concentration and therapeutic efficacy in breast cancer. For instance, a study demonstrated that USMC technology significantly increased the drug concentration and therapeutic efficacy of orally administered gefitinib in mice with ovarian cancer.[40] Additionally, the anti-tumor effects of PD-1 immunotherapy can be synergistically enhanced by modifying the local tumor microenvironment. This can be achieved by adjusting vascular permeability and inducing T-cell infiltration, thereby improving overall therapeutic outcomes.[126,127] This study introduces a novel therapeutic modality that combines low-intensity focused ultrasound-targeted microbubble disruption (LIFU-TMD) with programmed death-ligand 1 (PD-L1) blockade immunotherapy. LIFU-TMD has been demonstrated to induce the disruption of aberrant tumor vasculature, thereby reducing tumor blood perfusion and facilitating the transformation of the tumor microenvironment (TME). This transformation sensitizes the TME to anti-PD-L1 immunotherapy, resulting in significant inhibition of 4T1 mammary carcinoma growth in murine models.[121] The mechanistic underpinnings of this synergistic effect include the enhancement of CD8+ T cell infiltration within the tumor, alleviation of the immunosuppressive TME, and the induction of systemic anti-tumor immune responses. These effects collectively augment the local and distal therapeutic efficacy of anti-PD-L1 antibodies. Additionally, ultrasound-targeted microbubble destruction (UTMD) has been found to play a pivotal role in the optimization of chimeric antigen receptor T-cell (CAR-T) therapies. UTMD can precisely guide CAR-T cells to specific tumor sites, either through ultrasound-mediated delivery or by enhancing the infiltration and activation of CAR-T cells within the tumor. These mechanisms collectively improve the efficacy of CAR-T therapies.[128]

Ultrasound technology serves dual purposes in clinical settings, with significant applications in both therapeutic and diagnostic modalities. In the context of breast cancer diagnostics, ultrasound is an indispensable tool for the early detection of malignancies, particularly in regions such as China, where its usage is more prevalent than conventional mammography. This preference is attributable to the fact that most Chinese women possess small and dense breast tissue, which can obscure the sensitivity of traditional mammographic imaging. In the diagnostic workflow for breast cancer, ultrasound offers several advantages over radiography, especially in dense breast tissue. Ultrasound imaging provides

more detailed information about the internal structure and composition of the breast, enhancing the detectability of lesions that may be masked by dense tissue in mammograms. This capability is crucial for early intervention and improved patient outcomes.[129,130]

While the above presentation clearly showcases the significant clinical potential of UTMD in breast cancer immunotherapy, several challenges remain. These include the unpredictability of UTMD's interaction with adjuvant radiotherapy, chemotherapy, and other therapies; the potential for immune-related adverse reactions; and the intricacy of the tumor's anti-immune mechanisms. Consequently, further research and clinical trials are essential to refine these therapeutic strategies and to establish the optimal use of UTMD in breast cancer treatment.

UTMD in Hormonal Therapy of Breast Cancer

HER2+ breast cancer is a highly aggressive subtype, and precise assessment of HER2 expression status is crucial for determining patient eligibility for targeted anti-HER2 therapy.[131,132] While this treatment effectively reduces recurrence and mortality risks, it also presents various side effects.[133] Hormone therapy for breast cancer targets hormone receptor-positive (ER+/PR+) subtypes.[134] Moreover, microbubble-enhanced and ultrasound-guided (MB+US) drug delivery has demonstrated promise across various therapies, including HER2+ breast cancer, due to its tunable, noninvasive, and spatially targeted nature.[86,135,136] Researchers have developed ultrasound imaging combined with poly (lactic acid-hydroxyacetic acid) nanocarriers to dynamically monitor tamoxifen resistance.[137] This technology, which integrates hormone therapy with ultrasound to create targeted nanobubbles for ultrasound molecular imaging, offers a promising tool for real-time monitoring of drug resistance in the clinic. While these therapies have proven effective in clinical settings, challenges such as drug resistance and side effects persist.[138,139] UTMD has shown potential in managing side effects and providing new insights into drug resistance. Consequently, there is a need to continually explore and develop more effective treatment options for breast cancer, particularly in the application of ultrasound nanobubbles.

UTMD in Radiofrequency Ablative Therapy for Breast Cancer

Imaging-guided Radiofrequency ablation (RFA) of breast cancer has become an important tool in modern breast therapy[140,141](Figure 3). Among these techniques, ultrasound-guided RFA is a new minimally invasive breast surgical modality widely used in clinical practice. RFA generates heat locally by causing ionic oscillations through the high-frequency alternating current flowing around the electrodes, leading to protein denaturation and coagulative necrosis of tissues, as well as apoptosis and inactivation of tumor cells.[142–144] As a localized radical treatment, it offers the advantages of easy mastery, small incisions, and good cosmetic effects.[145] Currently, RFA has shown good results in the treatment of breast cancer. Early studies have confirmed that radiofrequency ablation can cause complete necrosis of ablated tumor cells in various types of breast diseases. The rate of complete tumor necrosis after ablation, as observed through pathological examination, ranges from 76% to 100%.[146,147] Subsequent studies have shown that the 5-year progression-free survival rate of patients who underwent radiofrequency ablation was significantly higher than that of patients who underwent post-conservative radiotherapy, with 5-year survival rates ranging from 87% to 97%.[148–150] Zhang et al combined RFA with lumpectomy, applying RFA to inactivate the peritumoral invasive cavity after the surgical procedure. This approach resulted in improved breast appearance, reduced local recurrence rates, and decreased secondary surgery rates.[151] The application of RFA in early breast cancer demonstrated good local control rates, safety, and high patient quality of life and satisfaction,[152] underscoring the clinical advantages of radiofrequency ablation in breast cancer treatment and the potential for local treatment of breast cancer tumors. Regarding the potential for widespread use of radiofrequency ablation in the treatment of malignant breast tumors, the key challenges currently faced are primarily twofold: efficiently and thoroughly ablating the tumor to achieve complete inactivation, and non-invasively detecting recurrence post-procedure.[153,154] In addressing these challenges, researchers have discovered that ultrasound, on one hand, induces rapid expansion and contraction of microbubbles within tissues, thereby generating localized high temperatures that augment the thermal effects of radiofrequency ablation and enhance its efficacy. On the other hand, the combination of ultrasound with targeted microbubbles composed of specific nanomaterials enables the visualization of specific tumor sites.[155,156] Consequently, recent studies have demonstrated that an approach combining ultrasound-targeted microbubbles with radiofrequency ablation techniques can enhance the therapeutic efficacy of

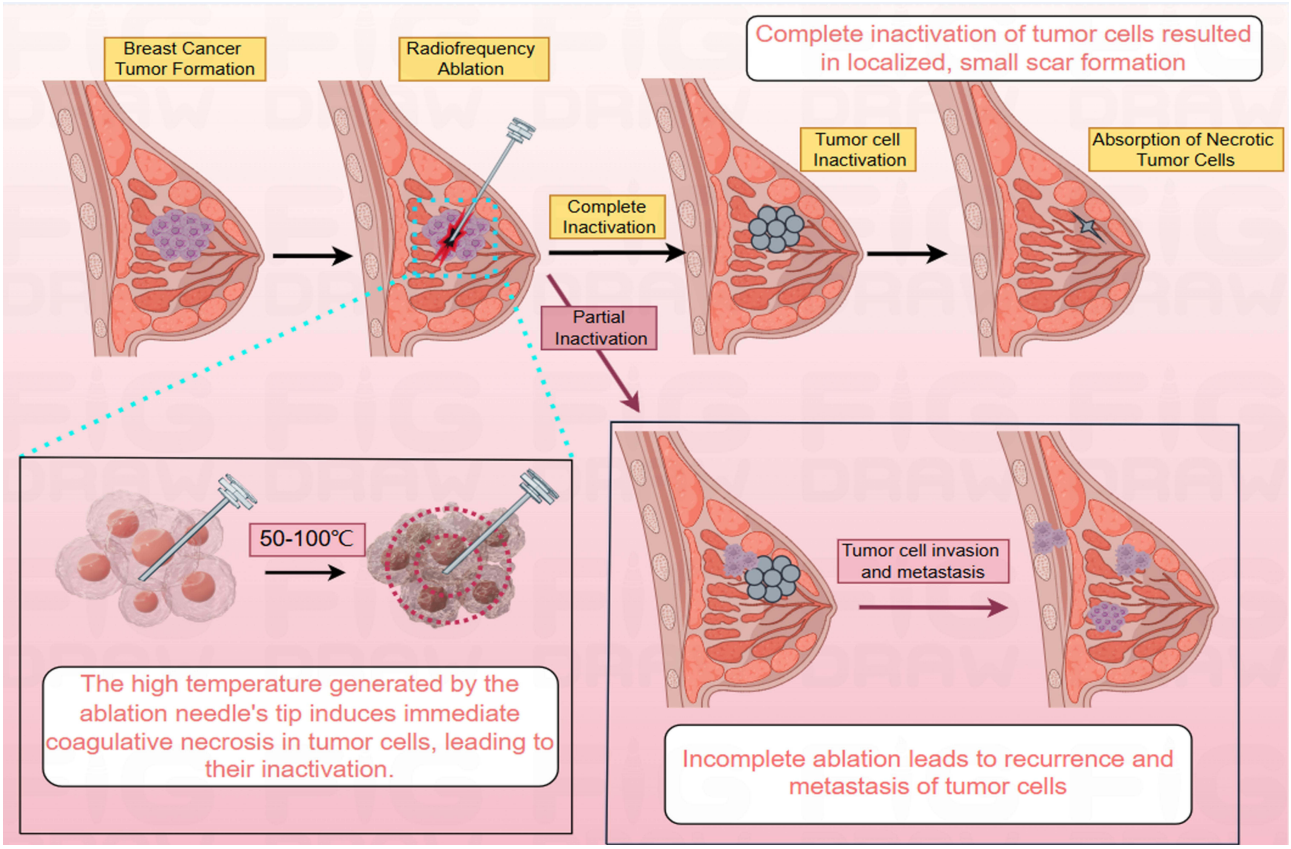

Figure 3 Influence of complete radiofrequency ablation on prognosis of breast cancer.

ablation.[157–159] It has been shown that the combined use of low-frequency ultrasound and microbubbles amplifies the inhibitory effect of radiofrequency ablation on pancreatic cancer cells, reducing cell migration and proliferation.[160] Furthermore, in a study on breast cancer ablation therapy, ultrasound-guided cryoablation combined with endocrine therapy in ER-positive, HER2-negative locally advanced breast cancer was found to be effective in improving ablation efficiency and promoting tumor cell apoptosis using this combined treatment method.[161] This further confirms that the combined effect of ultrasound microbubbles plays a positive role in enhancing ablation efficiency, thereby promoting tumor cell apoptosis and inactivation in ablation therapy. The underlying mechanism may be that microbubbles, under ultrasound irradiation, act as bioaugmentation agents, enhancing the efficiency of thermal ablation therapy by altering the acoustic impedance difference and thus increasing ultrasound energy deposition in the tissue environment.[162] Moreover, in another study, researchers discovered that perfluorocarbon (PFC) nanoparticles, when subjected to focused ultrasound (FUS), transform into microbubbles during tumor thermal ablation. This transformation alters the acoustic environment of the tissues and enhances ultrasound energy deposition, thereby achieving synergistic FUS-assisted ablation therapy for tumors.[84,163,164]

In a comparative trial, the study demonstrated that the strategy of combining ultrasound with microbubbles significantly improved the accuracy and efficacy of ablation, particularly in the treatment of breast cancer tumors, and showed great potential for use in breast cancer treatment. However, several issues require further investigation and optimization: Microbubble stability and safety: The materials and types of microbubbles used in the study need to be optimized to ensure in vivo stability and safety, and to minimize potential side effects. Equipment and parameter optimization: The equipment and parameter settings used for the ultrasound-microbubble combination need to be further optimized to achieve the best treatment outcomes. This includes optimizing the ultrasound frequency and power, as well as the microbubble concentration and size. Clinical validation: Although numerous studies are currently in the animal experimentation and theoretical research stages, large-scale clinical trial data are scarce. Consequently, further clinical

validation is required to evaluate the actual efficacy and safety of this approach in treating various tumor types and stages. The aforementioned review indicates that the application of ultrasound combined with microbubbles in radiofrequency ablation holds promise; however, it still necessitates continued exploration and optimization to play a more significant role in clinical practice.

Conclusion and Prospect

Ultrasound-targeted microbubble destruction (UTMD) has emerged as a potent, non-invasive adjuvant therapy for breast cancer treatment, demonstrating significant efficacy in enhancing the immune microenvironment and augmenting the effectiveness of radiotherapy, immunotherapy, and ablative therapies. This novel approach challenges the traditional paradigm, which has primarily focused on the direct cytotoxic effects of therapeutic agents on tumor cells. Despite the development of diverse therapeutic modalities, the lack of optimal delivery vehicles and strategies to improve the tumor microenvironment has hindered the full potential of these treatments. UTMD addresses this gap by enhancing therapeutic outcomes. To fully exploit the therapeutic potential of UTMD in breast cancer adjuvant treatment, several key areas of challenges and future research directions are identified. Optimizing ultrasound parameters, such as frequency and intensity, to maximize drug efficacy, and enhancing the biocompatibility and in vivo stability of microbubbles or nanocarriers to minimize side effects on normal tissues are crucial. Moreover, while preclinical studies and theoretical research have shown promise, there is a dearth of large-scale clinical trial data. More clinical validation is necessary to assess the efficacy and safety of UTMD across different types and stages of breast cancer. As the application of UTMD in breast cancer treatment continues to evolve, it is expected to play a pivotal role in the future of oncology. The academic community must rigorously investigate and refine the parameters and safety profiles of UTMD to ensure its maximum clinical benefit. By addressing these challenges, UTMD can become a cornerstone in the multidisciplinary approach to breast cancer treatment.

Data Sharing Statement

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

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