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Ultrasound-Based Radiation Enhancement: Concepts, Mechanisms and Therapeutic Applications

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Abstract

Microbubbles have emerged as versatile carriers used both for cancer diagnosis and therapy. Microbubbles in the presence of ultrasound waves undergo cavitation, generating bioeffects near the cell's vicinity. Studies have shown ultrasound-stimulated microbubbles (USMB) to cause mechanical perturbation of endothelial cells, resulting in acid sphingomyelinase (ASMase)-induced ceramide production. Disruption of endothelial cells further causes vascular deterioration, leading to secondary tumor cell death. These effects are known to be synergistically higher when USMB is combined with radiation. This paper provides insight into the use of USMB as a potential radioenhancer. The possible underlying mechanism and therapeutic effects of combining USMB and radiation therapy are also presented.

Keywords

cancer, cancer therapy, cancer treatment, cell death, cell signaling, radiation therapy

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Radiation therapy (RT) is one of the most commonly utilized treatments for cancer. Canonical radiobiology purports that high radiation doses destroy cancer cells by damaging their DNA.[1] Tumors in the patient's body are targeted either by external or internal RT. The former uses a radiation beam (typically X-rays, electron beams, or, less commonly, protons) to eradicate cancerous tumors, whereas the latter involves radioactive substances placed directly into the tumor site or in nearby regions to destroy cancer cells.[2] Despite the effectiveness of RT in treating different cancers, acute and long-term side effects caused by radiation toxicity cannot be avoided. Advances have been made to minimize such toxicities by incorporating multimodal therapy for greater treatment effectiveness. Studies have shown that concurrent systemic therapy (with chemotherapy or immunotherapy) can significantly increase the effectiveness of RT and help reduce the radiation doses required for treating cancers. However, some reports also suggest that the non-specific nature of chemotherapy may further elevate clinical toxicity.[3] Therefore, novel localized radiosensitizers or enhancers of radiation effect that can potentiate the efficacy of RT with minimal or no toxicities are still pertinent. Recent work has investigated the effects of other energy forms, such as ultrasound, in terms of mechanically inducing biological processes and biochemical changes, which can then enhance radiation effects.[4,5]

Extensive recent preclinical work suggests that ultrasound-stimulated microbubbles (USMB) can act as a potential radioenhancer.[4,5] Microbubbles are tiny bubbles that typically range between 0.5–10 μm in diameter. These are gas (typically perfluorocarbon, sulfur hexafluoride, or nitrogen)-filled bubbles that are stabilized within a shell composed of lipids, proteins, or polymers. They are widely used as ultrasound contrast agents

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for cancer diagnosis and recently have found experimental use for therapeutic purposes.[6] Upon interaction with the ultrasound waves, the bubbles start oscillating (stable cavitation). Depending on microbubble composition, particular pressures can cause bubble collapse and bursting (inertial cavitation). Cavitation-induced microstreaming and microjets or direct mechanical perturbation of endothelial cell walls cause bioeffects in the nearby surrounding tissues.[7] One of the most documented phenomena observed upon USMB exposure is the disruption of endothelial cells leading to blood vessel damage.[4,5]

Specifically, it has been demonstrated that either USMB treatment alone or treatment with high-dose RT (>8-10 Gy) can perturbate endothelial cell membranes causing the activation of the acid sphingomyelinase (ASMase) pathway - resulting in the release of ceramide, a lipid cell death signaling molecule. Either treatment leads to endothelial cell death *in vitro* or *in vivo*.[4,5,8,9] These effects amplify tumor responses synergistically, demonstrating at least 10-fold higher tumor cell death when USMB treatment is combined with RT.[4] Several *in vitro* and *in vivo* studies indicate that the combination of USMB and RT results in the rapid disruption of tumor vasculature (within 24 h), causing secondary tumor cell death leading to enhanced tumor response.[4,5,8,9] A study by El Kaffas *et al* investigated the effects of USMB and RT (2 or 8 Gy) in the activation of the ASMase-ceramide pathway using wild-type and ASMase knockout mice (C57BL/6) bearing fibrosarcoma tumors (MCA-129).[5] In addition, a group of wild-type mice were also treated with the ceramide inhibitor sphingosine-1-phosphate (S1P). Results indicated that the wild-type animals treated with a combination of USMB and RT demonstrated an approximately 40% decrease in tumor perfusion within 3 h following treatments.[5] Further, an increase in tumor cell death within 24 h with enhanced ceramide staining was also observed in the wild-type group, as confirmed by immunohistochemistry. However, the effects were found to be minimal in ASMase-knockout mice or S1P-treated mice.[5] Furthermore, the changes in the levels of various ceramide species following treatments were also evaluated. Blood plasma ASMase was found to be significantly higher in wild-type mice, whereas knockout mice demonstrated increased ceramide kinase levels, an enzyme responsible for phosphorylating ceramide to ceramide-1-phosphate (C1P).[5] The work concluded that the acute vascular shutdown caused within a few hours of treatment was responsible for the overall enhancement of radiation response.[5] Previously, Al-Mahrouki *et al* carried out an *in vitro* gene profiling study using endothelial cells (human umbilical vein endothelial cells (HUVEC)) to elucidate the bioeffects of USMB and RT (8 Gy).[8] Results indicated an up-regulation of UDP-glycosyltransferase 8 (UGT8), cytochrome c oxidase (COX6B1), and caspase 9, genes that are associated with ceramide cell death signaling.[8] A detailed *in vivo* study was later carried out focusing on the role of UGT8 in USMB and radiation-induced tumor response.[10] PC3 prostate tumor xenografts with up- and down-regulated UGT8 genes were included in the study. It was found that the combination of USMB and RT (8 Gy) caused a greater accumulation of ceramide in the down-regulated UGT8 group, leading to enhanced apoptosis with further diminished tumor blood flow and oxygen saturation observed in this group. On the other hand, minimal effects were seen in the up-regulated group.[10] Thus, the direct involvement and importance of ceramide cell death signaling and its impact on vessel damage causing overall tumor vasculature disruption, in addition to cell membrane effects on tumor cells secondary to endothelial cell disruption, was reported.[10]

Initial critiques suggest tumor responses to radiation involve diminishment in vascular content and hypoxia.[11] This has not borne out to be the case – the use of USMB with RT causes a massive ischemic event in the tumor (anoxia) to the point where tumor cells in large volumes are starved of oxygenation and rapidly die. This results in enhanced radiation responses and superior tumor kill as demonstrated in animal experiments *in vivo* where groups treated with USMB and RT demonstrate superior animal survival compared to groups receiving single modality treatments alone.[12,13] Tumor selectivity is attained by applying ultrasound in a conformal manner.

This work to date has involved multiple studies that included cumulatively over thousands of mouse-borne tumors and hundreds of rabbit-borne tumors and in clinical trials with human patients with breast, head and neck, and liver tumors.[4,5,12–19] The methodology has also been recently suggested to be used in CNS-borne tumors.[20–22]

Apart from the biophysical disruptions of vascular endothelium leading to enhanced tumor response caused by a combination of ultrasound microbubbles and radiation, the use of USMB and RT in the opening of the blood-brain barrier (BBB) has also been explored recently.[20,21] The BBB plays a key role in maintaining brain physiology and brain health. It comprises specialized microvascular endothelial cells that maintain brain hemostasis.[23] Surprisingly, the role of endothelial cell effects in regards to ceramide formation during BBB opening has not been explored. More specifically, the functioning of these cells when treating brain tumors or opening of BBB following focused ultrasound (FUS) and radiation has yet to be elucidated. A study carried out recently used microbubble-mediated FUS with combined 4 Gy to explore the effect of treatment in healthy rat brains and rats bearing F98 gliomas.[21] The results indicated apoptosis increased by 93% and 396% and vessel-associated ceramide increased by 320% and 336% in tumors treated with FUS and radiation.[21] Another study utilized FUS + 2 Gy radiation, demonstrating prolonged survival of animals bearing GL261 malignant glioma.[20] However, no histopathological analysis was reported in the study, which might be extremely important when it comes to determining the tumor architectural features as well as the biological behavior of cancers. Additionally, a recent preclinical study conducted using mice bearing diffuse midline glioma (DMG) demonstrated FUS combined with hypofractionated RT to be safe in the opening of BBB.[22] Intriguing results were reported regarding the feasibility of FUS and RT techniques. Moreover, the histopathological analysis compared normal tissue and tumor tissue, revealing apoptosis-like features

including cell swelling, vacuolar degeneration, and eosinophilic neurons with pyknosis following a combined treatment of FUS and radiation.[22] These results indicate that not only do FUS and RT open the BBB, but also suggest enhancement of the radiation effect.

Although the BBB disruption is found to be safe in most preclinical models and patients with glioblastoma (GBM), several questions remained unanswered, such as what happens to the brain microvascular endothelial cells during or after BBB opening in terms of ASMase-stimulation and ceramide formation. More detailed studies focusing on the involvement of such mechano-acoustical biological signal transduction pathways in BBB opening are needed. Also, studies investigating the potential mechanism of synergy between FUS and RT in BBB opening are urgently required. BBB-opening alone may be safe; however, when combined with RT, it may not be, and caution must be applied given established USMB effects on endothelial cells when combined with radiation.

Overall, USMB can be used as a potential radiosensitizer. When used together, USMB and RT can activate the ASMase-ceramide pathway, enhancing tumor response. Despite the promising outcomes of combining USMB and RT, which have mostly been studied in the short term, future studies should include the long-term therapeutic effects of these treatments.

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