

Non-Invasive Physical Stimulation to Modulate the Tumor Microenvironment: Unveiling a New Frontier in Cancer Therapy

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Abstract

The tumor microenvironment (TME) has a crucial role in tumor development, metastasis, and recurrence. The chaotic and complex physical structure of the TME not only limits drug delivery but also contributes to the development of resistance to immunotherapy. Breaking the physical barrier limitation of the TME could further optimize the existing tumor treatment protocols. Physical stimulation, such as ionizing radiation, light, electricity, magnetic field, and ultrasound, modulate the TME by altering tumor vasculature, remodeling the extracellular matrix, and activating immune responses to achieve the goal of adjuvant to other tumor therapeutic approaches. In addition to adjuvant chemotherapy and immunotherapy, these physical stimulations also enhance the efficacy of other physical treatments for cancer. In this review we discuss the structural characteristics of TME and focus on the modulation of TME by different physical stimulations. We also analyze the adjuvant effects of these stimulations on other tumor therapies.

Keywords

Adjuvant approach, drug delivery, extracellular matrix, immune responses, immunotherapy, tumor vasculature.

Introduction

The tumor microenvironment (TME) is a crucial factor that influences the survival, invasion, and metastasis of tumor cells. The TME consists of various components, including immune cells, stromal cells, blood vessels, and the extracellular matrix [ECM] [1]. The TME has a significant role in promoting cancer growth, metastasis, and resistance to treatment [2]. The dense ECM structure of the tumor tissue, along with the compressive stress on blood vessels, lymphatic vessels, and other tissues, impede drug delivery [3, 4]. Additionally, the biophysical structure of the TME can hinder immunotherapy-related antibodies or activate downstream signaling through mechano-transduction, leading to upregulation of programmed cell death ligand 1 (PD-L1) expression and anti-apoptotic molecules, the massive secretion of immunosuppressive factors, and abnormal function of anti-tumor immune cells. These effects act synergistically to result in resistance to immunotherapy [5].

In conventional tumor treatment regimens, chemotherapeutic agents have low specificity [6, 7]. As a systemic therapy,

the higher the dose of the chemotherapeutic drug, the greater the side effects [6, 7]. Physical stimulation, such as ionizing radiation, light, electricity, magnetic field, and ultrasound [8, 9], offer significant advantages in the treatment of TME limitations. Specifically, radiation or heat can break the physical barrier of the TME, which makes it easier for drugs to accumulate at the tumor site by modulating the structure of the TME and lowering the interstitial fluid pressure (IFP), thus reducing serious side effects and improving the efficacy of treatment [10]. In addition, emerging tumor treatments, such as cancer immunotherapy (CIT), have limited effects due to immune tolerance associated with the TME. Currently, the most successful and widely used immune checkpoint in clinical practice is PD-L1/programmed cell death protein 1 (PD-1). However, PD-L1/PD-1 monotherapy only has an effective rate of 10%–40% due to primary resistance [5]. In contrast, combining immunotherapy with physical stimulation therapies, such as radiation, photodynamic therapy (PDT), photothermal therapy (PTT), and sonodynamic therapy (SDT), significantly improves the efficacy of immunotherapy

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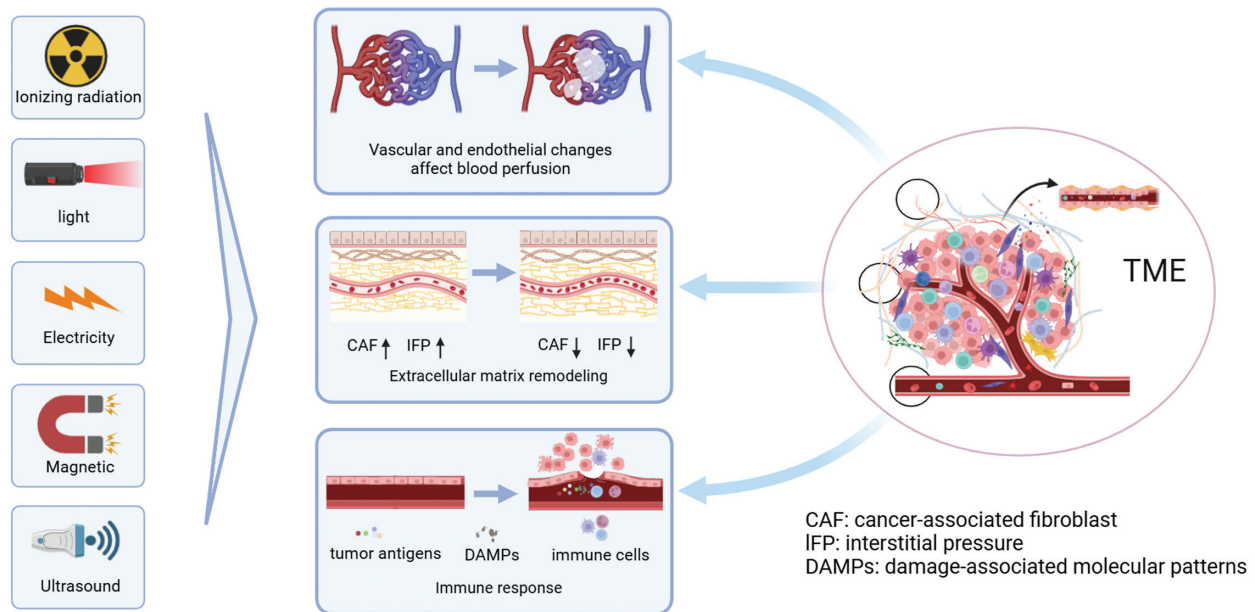


Figure 1 A schematic diagram of the tumor microenvironment (TME) modulation by physical stimulation. Under specific conditions, physical stimulation (ionizing radiation, light, electricity, magnetic field, and ultrasound) modulate the TME by altering the endothelium and vasculature to affect perfusion, reduce the interstitial fluid pressure (IFP), cause ECM remodeling, and activate immune responses to modulate the TME.

[11]. All of the combined therapeutic approaches demonstrate the advantages of physical stimulation in modulating the TME for drug delivery and adjuvant tumor therapy and show the future promise of the multidisciplinary intersection in medicine. In this review we explore the structural features of the TME and focus on the modulatory effects of different physical stimulations on the characteristic structures of the TME, such as blood vessels, the ECM, and immune cells (Figure 1). The adjuvant effects of these stimulations on other tumor therapies are also analyzed.

Characteristics of the TME

The TME refers to the internal physicochemical state that supports the survival of cancer cells. The TME encompasses various components, including blood vessels, the ECM, immune cells, stromal cells (e.g., fibroblasts), lymphatic vessels, soluble cytokines, mediators, and other non-cellular elements, such as extracellular vesicles [12, 13]. The characteristics of the TME include low acidity [14], H_2O_2 accumulation [15], hypoxia [16], low catalase activity [17], high reducibility, and immunosuppression [18]. The organizational structure and metabolic changes within the TME interact with one another. From the perspective of the tumor vasculature, compared to normal blood vessels, the vascular network at the tumor site is irregular and disorganized. Tumor cells are metabolically active, continuously consuming oxygen and nutrients, and secreting large quantities of pro-angiogenic factors, which further contribute to abnormal blood vessel growth. Simultaneously, the abnormal tumor vasculature contributes to the hypoxic environment within the TME. The aberrant tumor vessels also contribute to increased IFP, which promotes tumor

progression and immune resistance. The walls of these abnormal vessels contain a large number of endothelial cells, which serve as a significant source of cancer-associated fibroblasts (CAFs). Hypoxic conditions stimulate CAFs, leading to CAF dispersal and exacerbating physical stress on the tumor. As a result, blood and lymphatic vessels can be compressed, leading to reduced perfusion [19]. CAFs also secrete factors that impede T lymphocyte infiltration, reduce immune cell activity, and promote the accumulation of immunosuppressive cells, thereby suppressing anti-tumor immunity [20]. Furthermore, CAFs secrete factors that promote tumor angiogenesis [21]. In conclusion, the components of the TME interact with each other and collectively establish the tumor survival environment. Modulating tumor vascular changes and remodeling the ECM through physical stimulation create favorable conditions for drug and nanoparticle delivery. Moreover, physical stimulation of the TME activates the immune response, laying the foundation for combining relevant tumor therapy with immunotherapy in a physically stimulated approach.

Application of physical stimulation to the TME

The abnormal tumor vasculature within the TME has a crucial role, displaying structural and functional abnormalities that result in hypoxia, acidity, elevated IFP, and increased permeability. These factors not only sustain the TME but also facilitate tumor cell invasion, metastasis, and immunosuppression, while hindering drug delivery [19]. Physical stimulation can be used to target the tumor vasculature and address these issues. For example, radiotherapy has the potential to normalize the tumor vasculature at specific

dosage levels [22]. CAFs present within the ECM have a role in remodeling the ECM, which creates a supportive microenvironment for cancer cells and acts as a physical barrier that hinders drug penetration. Moreover, CAFs secrete matrix metalloproteinases (MMPs) that interfere with ECM degradation, which initiates cancer cell migration and invasion [12]. Innovative approaches, such as physical stimulation using nanoparticles that target CAFs and employing a low-frequency rotating magnetic field (RMF) with torque, have shown promising results in disrupting CAFs [23]. The remarkable efficacy of immune checkpoint inhibitors (ICIs) and chimeric antigen receptor T (CAR-T) cell therapy in solid tumors has prompted researchers to investigate the role of immune cells within the TME. Over the past decade, significant progress has been made in understanding the role of immune cells in the TME and developing strategies for immune cell-based tumor therapy [12]. PDT and PTT have been demonstrated to reprogram tumor-associated macrophages (TAMs), inducing activation of innate immunity by upregulating M1 macrophages and downregulating M2 macrophages [24]. The vasculature, ECM, and immune responses within the TME are modulated by several types of physical stimulations (ionizing radiation, light, electricity, magnetic field, and ultrasound; **Table 1**).

Ionizing radiation

Radiotherapy is a crucial component of cancer treatment, with >50% of cancer patients undergoing at least one session of radiotherapy during treatment. Radiotherapy utilizes high-energy ionizing radiation, such as γ -, β -, and X-rays, to target and damage tumor cells. The primary objective of radiotherapy is to induce DNA damage directly in tumor cells or indirectly generate reactive oxygen species (ROS) by interacting with water molecules, which leads to the elimination of tumor cells [25].

Vasculature alteration

The TME exhibits distinct physical characteristics, including ECM structure and stiffness, solid stress, the IFP, and

vascular shear stress [5]. These factors contribute to tumor progression and resistance to immunotherapy through various mechanisms. X-ray radiation remodels the stroma and alters tumor-associated blood flow, while also reducing the IFP by impacting tumor cell morphology, intra-tumoral microvasculature, and mesenchyme, thereby leading to vascular decompression and improved perfusion [26]. Pre-existing alterations in the tumor vasculature often hinder T cell infiltration into the TME due to the endothelial barrier. However, radiation induces increased expression of adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 [27], which promotes activation of endothelial-type nitric oxide synthase (eNOS) and nitric oxide (NO) production [28]. This process induces angiogenesis, enhances tumor blood flow [29], and facilitates T cell homing by upregulating E- and P-selectin expression on endothelial cells [30]. The effects of radiation on endothelial cells and blood vessels are dose-dependent (**Figure 2**).

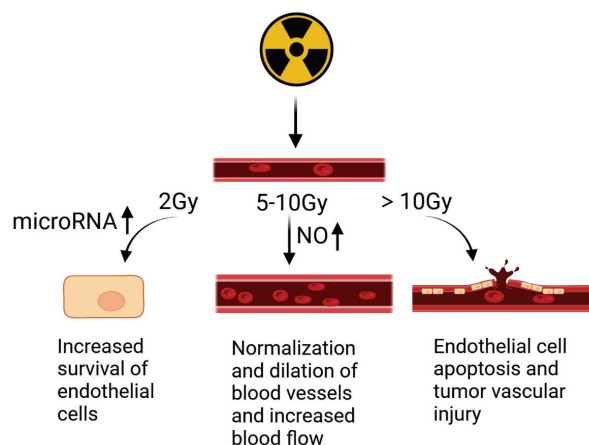


Figure 2 Effects of different radiation doses on tumor vasculature and endothelium. At a radiation dose of 2 Gy, microRNA upregulation increased endothelial cell survival [136], normalization and dilation of the tumor vasculature and increased perfusion were observed at radiation doses of 5-10 Gy [22], and apoptosis of endothelial cells and damage to the tumor vasculature were induced at radiation doses >10 Gy [22].

Table 1 Summary of the Effects of Different Physical Stimulations on the Vasculature, ECM, and Immune Responses

Physical Stimulation	Effects on the TME		
	Vasculature	ECM	Immune Responses
Ionizing radiation	Normalization, dilation, and collapse of tumor vasculature [22]	Matrix protein hydrolysis [34–37]	Induce ICD and reprogram TAMs [42, 43]
Light	Vasoconstriction/diastole and vascular collapse [56]	Denaturation of collagen [72]	Induce ICD and reprogram TAMs [24]
Electricity	Endothelial damage, vascular destruction, or vascular normalization [86, 87]	Activation of MMPs, a decrease in collagen production [89]	Induce ICD and re-program TAMs [90, 91]
Magnetic field	Inhibits tumor vasculature and promotes normal angiogenesis [96–98]	Collagen denaturation, CAFs death [23, 100]	Induce ICD and re-program TAMs [104, 105]
Ultrasound	Endothelial damage, vasodilation/constriction, vascular disruption, or generation [117, 121]	Decreased collagen content and decreased IFP by ultrasound combined with MBs [126, 127]	Induce ICD and re-program TAMs [130, 131]

Abbreviations: MMPs: metalloproteinases; CAFs: cancer-associated fibroblasts; IFP: interstitial fluid pressure; ICD: immunogenic cell death; MBs: microbubbles; TAMs: tumor-associated macrophages.

Extracellular matrix remodeling

It is generally believed that CAFs actively promote cancer invasiveness by modulating various processes, including angiogenesis, inflammation, and ECM remodeling [31, 32]. CAFs do not undergo apoptosis at a radiation dose of 30 Gray (Gy), but senescence occurs when the radiation dose exceeds 10–12 Gy [33]. Radiation can affect ECM remodeling in tumors by modulating protease activity. Radiation has the potential to induce hydrolysis of matrix proteins within the ECM, releasing stored active molecules, such as angiogenic factors, growth factors, and active matrix components. Tumor cell protease activity is altered after radiation with MMP-2 expression upregulated in different types of tumors, such as lung cancer [34], pancreatic cancer [35], colorectal cancer [36], and glioblastomas [37], potentially leading to increased tumor invasion. Inhibition of MMP-2 before radiation enhances the sensitivity of lung tumor cells to radiation therapy. Furthermore, radiation affects other proteases, such as MMP-9, which undergoes altered expression and activity in hepatocellular carcinoma cells through the PI3K/Akt/NF- κ B cascade [38]. In non-small-cell lung cancer cells, a radiation dose of 2 Gy activates the SDF-1/CXCR-4 pathway, resulting in increased invasiveness through the PI3K/Akt and MAPK pathways, leading to MMP expression both *in vitro* and *in vivo* [39]. However, when administered at ablative doses, radiation upregulates MMP-3 and downregulates MMP-1, inducing premature senescence of CAFs and inhibiting proliferation, migration, and invasive capabilities [33]. Additionally, radiation-induced ECM remodeling involves lysyl oxidase (LOX), which enhances the soluble deposition and tensile strength of the ECM and correlated with tumor metastasis and invasion [40].

Overall, radiation may affect the invasive and metastatic capacity of tumors by modulating protease activity and ECM remodeling. Some differences exist as a function of tumor type and microenvironmental conditions, so further investigation is warranted to better understand the effects of radiation on the TME.

Immune response activation

Radiation can induce tumor-targeted immune responses, which are largely dependent on the antigenicity of tumor cells and the ability to generate adjuvant signals [41]. Specifically, radiation-induced immunogenic cell death (ICD) [42] contributes to immune cell dissemination. The effects of radiation dose on the TME are diverse. For example, low-dose radiation (2 Gy) stimulates TAMs of the M1 phenotype to produce inducible nitric oxide synthase (iNOS), which promotes the formation of an immunogenic TME and an immunogenic environment [43]. Conversely, higher radiation doses lead to pro-tumorigenic M2 phenotype TAM infiltration into the tumor [44]. Studies have indicated that a single radiation dose of 5–10 Gy results in mild vascular alterations within the TME. However, doses exceeding 10 Gy cause endothelial cell death, which leads to significant vascular damage and reduced blood flow. This

compromised vasculature hinders the recruitment of effector T cells, resulting in decreased immune cell infiltration and potentially contributing to hypoxia within the TME [45]. High-dose daily fractionation (8 Gy \times 2) has been reported to offer several advantages over low-dose daily fractionation (2 Gy \times 10). High-dose daily fractionation preserves peripheral and tumor-infiltrating effector immune cells, down-regulates immune-suppressive cells, increases immune cell expression in the TME, and enhances tumor-specific immune responses [46]. Radiotherapy is promising for adjuvant immunotherapy. For example, Qu et al. [47] failed to achieve the desired therapeutic effect when treating rectal squamous cell carcinoma with high PD-L1 expression by chemotherapy and immunotherapy alone, but after adjuvant radiotherapy, the tumors achieved complete remission with a recurrence-free status within 12 months.

Light

Phototherapy is a tumor treatment modality that primarily involves two techniques (PDT and PTT). Compared to traditional cancer treatment options (radiotherapy and chemotherapy), phototherapy specifically targets desired cells or tissues, resulting in improved targeting and reduced side effects [48]. PDT is a non-invasive therapeutic approach that utilizes photosensitizers (PSs). These PS agents selectively accumulate in tumor tissues. When exposed to a specific wavelength of laser light, the PS is activated, leading to the generation of ROS by consuming molecular oxygen. This process causes DNA damage and ultimately leads to tumor cell death [49]. With respect to light dosimetry, the used impact rate in PDT is typically kept low (<200 mW/cm²) to prevent thermal damage to the tissue [50]. It is also important to note that the individual components of PDT are non-toxic and it is only when PS is irradiated with light that cytotoxic reactive oxygen species (ROS), such as mono-linear oxygen (¹O₂), superoxide anion (O₂⁻), and hydroxyl radical (\bullet OH), are produced, which then cause cellular DNA damage [51].

Conventional PTT utilizes the heat generated by near-infrared materials (>50°C) to directly destroy tumor cells [52]. Compared to conventional treatments, PTT is less harmful to normal organs because PTT specifically localizes to tumor areas where PS accumulates and precisely applies laser irradiation. PTT is becoming a popular method for various diseases and cancers due to safety and precision [53]. However, the high temperatures of conventional PTT inevitably cause non-specific thermal damage to the surrounding healthy tissues. The necrosis induced by conventional PTT leads to severe local inflammation, further damage to healthy tissues, and even an increased risk of tumor metastasis [54]. Studies have also shown that conventional PTT suppresses host anti-tumor immunity by compromising immune antigens in the TME due to hyperthermia [55]. In contrast, mild PTT (mPTT [usually 42–45°C]) is a relatively low-temperature PTT method that offers advantages and features in terms of a modulating effect on the TME compared to conventional high-temperature PTT.

Vasculature alteration

PDT and PTT have dose-dependent effects on the vasculature. Early studies demonstrated that PDT causes multiple vascular effects, including altered vascular permeability, vasoconstriction/diastole, and vascular collapse [56]. In addition to direct damage to blood vessels, PDT also alters blood vessel permeability, which is crucial for systemic drug delivery. Studies have shown that when combined with medium doses of vascular-targeting agents, PDT significantly disrupts tumor perfusion and enhances drug delivery [57]. PDT has proven to be an effective treatment for a wide range of cancers, such as skin [58], head and neck [59], and superficial bladder cancers [60]. However, solid tumor hypoxia, which results from uncontrolled tumor growth and dysregulated angiogenesis, poses a challenge to PDT efficacy [61]. In addition, PDT leads to microvascular collapse, impeding O_2 transport and exacerbating hypoxia in the TME, which further diminishes the effectiveness of PDT [62]. To address this issue, a commonly used approach is to design a variety of smart nanoplateforms based on the high expression of H_2O_2 in tumor cells. Under laser stimulation, nanomaterials, such as MnO_2 [63], CaO_2 [64], RuO_2 [65], Fe_3O_4 [66], carbon dots [67], and biological catalase [68], react with H_2O_2 to produce O_2 , thereby alleviating hypoxia at the tumor site and improving the efficacy of PDT.

An early study [69] indicated that mild heat therapy enhances tumor blood flow and improves intravascular hemoglobin (Hb) oxygen saturation within the tumor. However, at higher temperatures, there is a transient increase in blood flow during the heating process, followed by the onset of vascular damage. The vascular damage leads to reduced tumor perfusion and oxygenation, decreased pH levels in the TME, and ultimately ischemia and cell death. Therefore, in addition to the development of nanoplateforms, mPTT also assists in improving the efficacy of PDT by increasing tissue oxygen saturation and ameliorating hypoxia in the TME.

In addition, the generation of ROS during PDT causes cellular DNA damage, whereas thermotherapy denature proteins involved in DNA repair [56] and thermotherapy has been shown to increase mitochondrial ROS levels [70], suggesting that PTT has a promising application in enhancing PDT.

Extracellular matrix remodeling

Solid tumors often exhibit increased ECM content, which contributes to elevated tissue stress and IFP. This finding, coupled with collapsed tumor blood vessels, can impede systemic drug delivery and compromise therapeutic efficacy [71]. In 1987 Barr et al. [72] conducted experimental studies in rats to determine the impact of PTT on the ECM. Barr et al. [72] used a 675-nm laser at 500 mW for 100 seconds on the rat colon, which resulted in an increase in temperature to $66 \pm 7.5^\circ C$. Transmission electron microscopy revealed significant swelling and structural changes in submucosal collagen, indicating thermal damage and protein denaturation. In contrast, when the colon was treated with a hematoporphyrin derivative, PDT (100 mW power for 500 seconds), resulted in no ECM structural changes. This finding suggested that

PDT preserves ECM structure (specifically, the submucosal collagen). Subsequent studies have also demonstrated that PTT with a near-infrared (NIR) laser or mild hyperthermia leads to collagen denaturation. Indeed, several studies have demonstrated that PTT or mild hyperthermia ($43^\circ C$ for 15 min) leads to collagen denaturation [73, 74]. Overall, these experimental findings indicate that with the assistance of some nanoparticles, PTT induces corresponding structural changes in the tumor ECM, including collagen reorganization. This facilitates more effective drug penetration into the tumor and contributes to an improved tumor treatment.

In 2023 Overchuk et al. [56] conducted a study on sub-therapeutic prostate-specific membrane antigen (PSMA)-targeted PDT ($50 J/cm^2$) in subcutaneous prostate tumor xenografts. The findings revealed a 2-fold reduction in the total collagen density of the tumors compared to the previous study and electron microscopy showed a sub-endothelial region with reduced collagen coverage. Due to the precise therapeutic boundaries and non-thermal nature, PDT preserves ECM structures, which aids in healing and reducing scarring. Both PDT and PTT induce changes in the ECM of the tumor, which can be beneficial in the therapeutic regimen.

Immune response activation

PDT and PTT elicit immune responses by releasing tumor-specific antigens (TSAs) and producing immune-modulating molecules, such as calreticulin (CRT), high-mobility group box 1 (HMGB1), ATP, and heat shock proteins (HSPs). These immune responses have a crucial role in targeting and eliminating tumor cells [75–77]. By inducing ICD, PDT and PTT lead to the release of inflammatory cytokines, such as IL-6, IL-1 β , TNF- α , and chemokine C-X-C ligand 2 (CXCL2). This initial inflammation promotes the activation and recruitment of antigen-presenting cells (APCs), such as dendritic cells (DCs), which can enter local lymph nodes, take up and process TSAs, present TSAs to naive T cells, thereby activating long-term adaptive immunity [78]. PDT and PTT treatments induce ICD and reprogram TAMs, promoting innate immunity by upregulating M1 macrophages and downregulating M2 macrophages (Figure 3). This re-programming boosts anti-tumor immune responses and contributes to the therapeutic effects of PDT and PTT in cancer treatment [24]. However, unlike PDT, PTT may only induce ICD within a specific thermal window. For example, a study conducted by Sweeney et al. [79] demonstrated that PTT using Prussian blue nanoparticles (PBNPs) in neuroblastoma cell-induced ICD at the optimal thermal dose. The expression of ICD markers, such as the release of calreticulin, ATP, HMGB1, and CRT, is most evident on the cell surface when the cells are heated at temperatures ranging from 50 – $60^\circ C$ for 10 min. Temperatures $<50^\circ C$ or $>60^\circ C$ were shown to be ineffective [75]. While some PDT regimens, particularly repeated PDT treatments, have shown the ability to activate adaptive immunity and induce effects *in vitro* alone [80], PDT and PTT typically require additional immune adjuvants or methods to enhance the immune response. Ghosh et al. [81] combined chemophototherapy and immunotherapy (a combination of

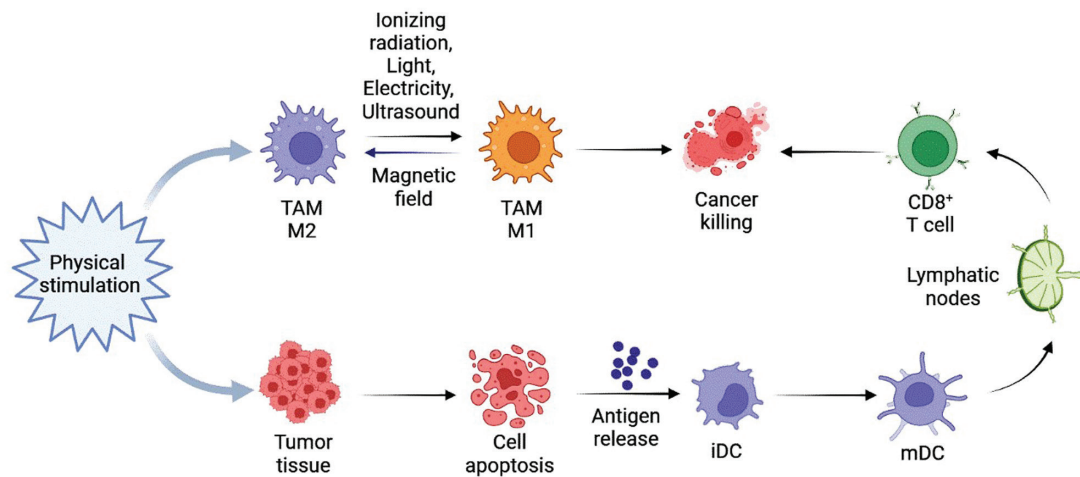


Figure 3 Diagram of the role of physical stimulation in the modulation of the tumor immune microenvironment. Except for magnetic fields, which polarize macrophages toward the M2 phenotype, other physical stimulations, such as ionizing radiation, light, electricity, and ultrasound, polarize macrophages toward the M1 phenotype. iDC: immature dendritic cell; mDC: mature dendritic cell.

PD-1 and anti-cytotoxic T-lymphocyte-associated protein 4 [CTLA-4] antibodies) to ablate medium-sized KPC pancreatic cancer tumors and induce memory immune responses. The results therein indicate that chemotherapy and immunotherapy alone cannot eliminate small KPC tumors. However, when used in combination, chemotherapy and immunotherapy can even clear medium-sized KPC tumors and protect against tumor re-attack. The enhanced synergy of these two treatments is facilitated by the penetration and accumulation of PDT-induced anti-PD-1 antibodies breaking through the physical barrier of the TME at the tumor site.

Electricity

Electric fields can have various biological effects, including stimulating healing, causing direct tissue damage, or inducing cell death by disrupting cell mitosis. In the field of electric field applied oncology therapy, pulsed electric field (PEF)-based therapies include irreversible electroporation (IRE), gene electrotransfer (GET), electrochemistry (ECT), calcium electroporation (Ca-EP), and tumor-treating fields [TTF] [82]. Among these therapies, IRE is widely utilized for tumor ablation. IRE involves the application of short pulses of high-pressure electricity to create nanoscale perforations in cell membranes, subsequently inducing apoptosis [83]. In recent years, TTF has shown unique advantages in the treatment of glioblastomas, pleural mesotheliomas, and lung and pancreatic cancers [82]. Studies have indicated that the use of low-voltage, mid-frequency (100-500 kHz) electric fields, such as TTF, impede DNA repair, induce autophagy, promote anti-tumor immunity, inhibit tumor cell migration and invasion, and alter the permeability of cell membranes and the blood-brain barrier [84].

Vasculature alteration

It has been shown that PEFs inhibit angiogenesis in tumor tissues and suppress tumor angiogenesis, leading to a reduction

in tumor growth [85]. In the study by B. Markelc et al, significant alterations in the morphology of endothelial cells were observed 1 h after application of an electric pulse. These cells became rounded and swollen, resulting in a constriction of the blood vessel lumen [86]. Other studies reported that ECT targets endothelial cells, triggering apoptosis and causing vascular destruction. Interestingly, electroporation exhibits greater efficacy in eliminating endothelial cells within small tumor vessels, while larger vessels appear to be more preserved [87]. Furthermore, electrical stimulation also breaks intracellular bioelectrical homeostasis, thereby promoting normalization of the tumor vasculature and facilitating drug delivery [88]. Li et al. [88] demonstrated that the combination of radio-stimulation and the chemotherapeutic agent, adriamycin, exhibits 1.8-fold higher anti-tumor efficacy compared to treatment with adriamycin alone.

Extracellular matrix remodeling

In 2022 Gouarderes et al. [89] demonstrated that PEFs induce remodeling of the ECM by activating MMPs and reducing collagen production. Regardless of the electrical stimulation protocol used in the experiments, the tissue collagen content was significantly reduced by 35%–50% after 1 week of electrical stimulation. PEFs promote collagen remodeling by transiently decreasing collagen production and increasing collagen degradation through sustained activation of MMPs by ROS. PEFs downregulate the expression of TGF- β , a major regulator of fibrosis, at the mRNA and protein levels. Furthermore, there was a substantial decrease in the gene expression of key enzymes involved in ECM cross-linking, such as lysyl oxidase (LOX) and transglutaminase.

Immune response activation

Electrical stimulation, like other physical stimulations, has the potential to directly eliminate tumor cells and elicit immune responses, making electrical stimulation a

promising therapeutic approach for tumors [90]. Research has indicated that tumor cell death induced by electrical stimulation undergoes conversion from non-immunogenic to immunogenic, which is known as ICD. This process leads to the massive release of tumor antigens and damage-associated molecular patterns (DAMPs), attracting DCs to gather at the tumor site [91]. Furthermore, DCs are responsible for presenting tumor antigens to T cells, thus triggering an adaptive immune response [92]. Kong et al. [90] demonstrated that driving local charge release under ultrasound irradiation significantly enhances M1 polarization in macrophages. Additionally, electrical stimulation induces inflammation, prompts the release of pro-inflammatory cytokines and chemokines, and stimulates anti-tumor immunity. For example, Chen et al. [93] demonstrated that TTF generates pro-inflammatory cytokines and type I interferon.

Magnetic field

Magnetic fields are classified based on their characteristics and generation methods. With respect to characteristics, there are constant magnetic fields (CMFs) and dynamic magnetic fields (DMFs). Regarding the generation method, magnetic fields can be categorized as alternating magnetic fields (AMFs), geomagnetic fields (GMFs), pulsating magnetic fields (PuMFs), and pulsed magnetic fields [PMFs] [94]. Magnetothermal therapy (MHT) is a technique that utilizes the thermal effect of magnetic nanoparticles (MNPs) to treat tumors. In addition to the thermal effects, magnetic field exposure generates corresponding mechanical forces, including tension, compression, shear force, and torque. These forces significantly impact the cellular environment, including the plasma membrane and internal organelles (e.g., mitochondria, lysosomes, and nuclei). This impact results in noticeable morphologic changes and intracellular damage. Activation of apoptotic or non-apoptotic cellular signals subsequently induces tumor ablation [8].

Vasculature alteration

At the vascular level, sub-thermal therapy has been shown to increase permeability of the tumor vasculature and enhance blood flow through the tumor vasculature. Nanoparticle extravasation increases with rising temperatures from 40°C–42°C. However, temperatures >42°C lead to bleeding and stagnation of the tumor vasculature [95]. A static magnetic field (SMF) reduces blood flow and platelet adhesion in tumor microvessels, thereby inhibiting tumor angiogenesis in murine experiments [96]. DMFs have both generative and inhibitory effects on angiogenesis. The specific effects of DMFs on angiogenesis vary depending on various parameters, such as magnetic field strength and frequency. Hu et al. [97] conducted a study reporting a strong inhibitory effect of time-varying magnetic fields on tumor growth. The effect was more pronounced at weak magnetic fields (1–5 nT) compared to strong magnetic fields (2–5 mT). However, DMFs may promote angiogenesis in normal tissues and pulsed

electromagnetic fields (PEMFs) with specific parameters have been shown to promote angiogenesis [98].

Extracellular matrix remodeling

Elevated temperatures have an impact on the ECM, and specifically on the structure of collagen [73, 99]. Magnetothermal therapy, similar to PTT, induces collagen denaturation and ECM remodeling. For example, when magnetic nanoparticles are positioned on top of collagen in the presence of AMF, the heat generated by the nanoparticles causes collagen to undergo phase change and melt [100]. Disruption of intracellular membranes has also been shown to effectively induce cell death [101]. Building upon this concept, Lopez et al. [23] found that nanoparticles specifically designed to target CAFs in pancreatic cancer are exposed to a low-frequency RMF, which resulted in interaction with lysosomal membranes. This interaction, facilitated by mechanical forces or activation of mechanosensitive ion channels on the lysosomal membranes, generated torque inside the lysosomes, effectively disrupting the lysosomal membranes and inducing death in CAFs.

Immune response activation

It has been shown that local heat therapy enhances anti-tumor immunity. Heat stress applied to tumor cells induces the release of HSPs that are recognized and activated by APCs [102]. This activation triggers the presentation of antigens to T cells, initiating adaptive immune responses [103]. In murine experiments, the use of magnetorheological fluid (MRF) or magnetotherapy to destroy primary tumors results in selective necrosis of malignant cells, while preserving tumor-infiltrating immune cells and inducing ICD. This *in situ* tumor injury activates DCs, recruiting DCs to the primary tumor site. The activated DCs then stimulate CD8⁺ T cells, leading to anti-tumor effects at the primary tumor site and distant sites [104]. It has also been shown that macrophages tend to be converted to a pro-tumor development M2 phenotype under high-gradient magnetic fields [105] [Figure 3].

Ultrasound

Ultrasound-related treatments have become widely used in the biomedical field due to safety, visualization capabilities, non-invasiveness, and relatively low cost of instruments [106]. Ultrasound waves are mechanical sound waves with frequencies higher than the human hearing range (16–20 kHz) that are capable of penetrating tissues up to a depth of approximately 10 cm [107]. Ultrasound waves enable precise localization of specific areas, selective destruction of pathologic tissues, and minimal damage to adjacent normal tissues and organs [108]. With respect to specific applications, therapeutic ultrasound can be categorized into non-thermal and thermal ultrasound energy, represented by SDT and high-intensity focused ultrasound (HIFU), respectively [109]. SDT activates the acoustic

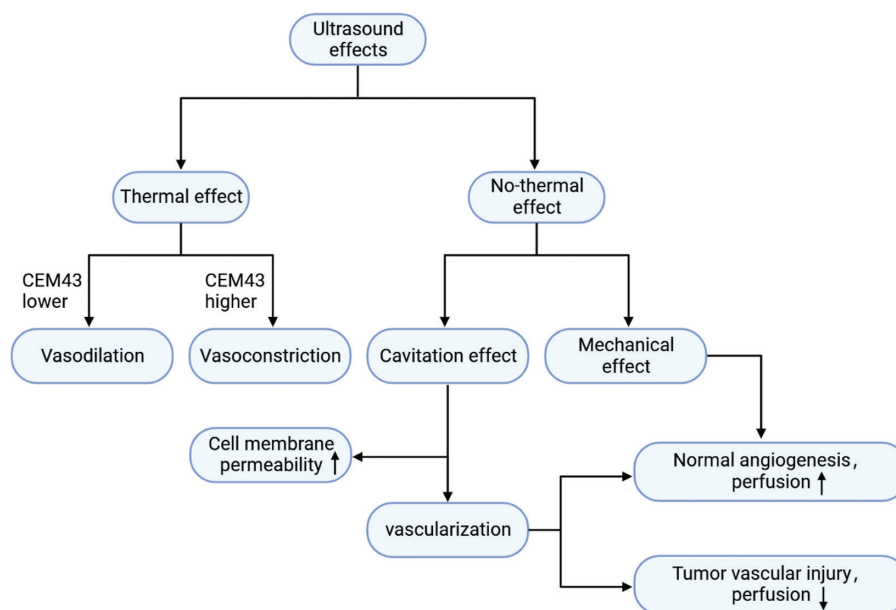


Figure 4 Ultrasound effects on blood vessels.

sensitizer through low-intensity ultrasound, leading to the production of ROS and subsequent destruction of tumor cells. As a non-invasive treatment for tumors, SDT shows promise as an anti-cancer therapy. The development and widespread utilization of nanomaterials has opened up opportunities for novel ultrasound sensitizers with tumor-targeting specificity. These sensitizers penetrate deep into the tumor, thereby improving the TME. HIFU, as a non-invasive procedure for cancer ablation, has also rapidly evolved in the treatment of solid tumors over the past few decades.

In addition, ultrasound induces a range of biological effects by activating acoustic sensitizers, including the generation of ROS [110], non-thermal effects, such as cavitation and mechanical effects, as well as thermal effects [111]. The thermal and mechanical effects of ultrasound have distinct applications in modulation of the TME.

Vasculature alteration

Hyperthermia induces several changes in the vasculature, with vasodilation being the most prominent. Such changes in the vasculature leads to increased blood flow, reduced hypoxia in the TME, improved acidity levels, and decreased interstitial pressure [112, 113]. Due to the direct reflection of the temperature receptor to activate the vascular smooth muscle, when the cumulative equivalent min at 43°C (CEM43) is low, the thermal effects of ultrasound result in vasodilation [114] and increase blood flow. Mild hyperthermia leads to a reduction in tumor IFP [115]. The effects of mild hyperthermia on blood vessels are reversible and do not cause tissue damage. Nevertheless, higher levels of CEM43 temporarily or permanently constrict blood vessels and cause congestion at the edges [106]. High-intensity ultrasound pulses also temporarily or permanently reduce blood vessel diameter. In addition to thermal effects, focused ultrasound (FU) transiently increases vascular permeability through mechanical

effects [106] [Figure 4]. Price et al. [116] demonstrated that the cavitation effect can cause microvascular rupture, leading to the extravasation of red blood cells into the ECM. This finding indicates that ultrasound-induced cavitation disrupts endothelial cell membranes and enhances cell membrane permeability [117]. Moreover, other mechanisms have been proposed to explain the effect of cavitation on cell membrane permeability. For example, cavitation leads to the formation of intracellular ROS, which might contribute to increased permeability of cell membranes [118]. When microbubbles/nanobubbles (MBs/NBs) collapse, the localized transient warming to 4300–5000 K affects mobility of the phospholipid bilayer and enhances cellular permeability [119]. Increased endothelial cell permeability following ultrasound combined with MB treatment induces blood-brain barrier opening [120]. In addition to the effect on cell membrane permeability, the cavitation effect affects the vasculature and blood perfusion. Blood perfusion is reduced shortly after exposure to FU and MBs, and may be due to the increased fragility of newly formed blood vessels in tumors compared to healthy tissues. When exposed to cavitation MBs, these fragile tumor blood vessels sustain vascular injury and disruption of the capillary walls, leading to reduced perfusion [121]. Additionally, when combined with MBs, FU open up blood vessels and enhance perfusion. This effect may be mediated by the release of NO triggered by shear stress. The effect could also be related to increased mechano-transduction within endothelial cells due to mechanical interactions [122]. In conclusion, the impact on perfusion depends on various factors, such as the specific ultrasound treatment used, the type of tissue, and the vasculature structure.

Extracellular matrix remodeling

Tumors typically exhibit elevated pressure, which can be classified as solid pressure and IFP. Solid pressure refers

to the pressure exerted by tumor cells, stromal cells, and ECM components as the density increases within the confined space of the host tissue. This solid pressure compresses pliable structures, like tumor blood and lymphatic vessels. Compression of lymphatic vessels reduces tumor drainage, leading to an increase in IFP [123]. Dysfunction of the lymphatic system and the dense ECM in tumors further exacerbates the increase in IFP. Exudate infiltrates from hyperpermeable vessels into the tumor interstitium, but due to the impaired lymphatic drainage and resistance posed by the dense ECM, the fluids are unable to adequately drain or penetrate the surrounding normal tissue [124]. Consequently, the tumor mesenchyme accumulates excess fluid that cannot be eliminated and the IFP gradually increases and eliminates the pressure gradient with the fluid, thereby limiting drug movement by convection. Reducing solid pressure and IFP to modulate the TME structure has been shown to improve drug delivery to the tumor mesenchyme [125].

Pulsed-HIFU remodeled the ECM in a murine A549 lung cancer experiment, resulting in increased vascular blood flow, decreased collagen content, and enhanced tissue permeability [126]. Because the size of MBs (2–3 μm in diameter) is limited by the vascular system, most MBs do not enter the ECM but how the size of MBs affects the tumor ECM is not clear. Xiao et al. [127] demonstrated a reduction in the IFP and an increase in drug penetration by a combination of 1 MPa and 10-min exposure time in ultrasound and MBs. To observe a reduction in the IFP, the presence of MBs is necessary because the ultrasound alone does not alter the IFP [128]. It is important to note that prolonged exposure to the ultrasound for >5 min destroys all MBs. Therefore, the observed decrease in the IFP reported by Xiao et al. [127] may have been primarily caused by hyperthermia rather than the direct effect of ultrasound on the IFP. Hyperthermia induced by ultrasound, which raises the body temperature to 42°C for 5 min, has been shown to reduce the IFP [115]. In the mentioned study [115], the authors also observed a reduction in the IFP after euthanasia in mice following ultrasound exposure. This finding suggests that the decrease in IFP was not solely a result of changes in blood flow but might also be associated with alterations in the ECM. Thus, the effects of ultrasound on the IFP may be attributed to a combination of factors, including changes in blood flow and potential modifications in the ECM.

Immune response activation

Ultrasound thermotherapy, ablation, tissue sectioning, and microbubble stabilization/inertial cavitation alter the TME, enhance immune activation, and inhibit tumor growth. Microbubble cavitation increases vascular permeability, which improves the delivery of immune cells, cytokines, antigens, and antibodies to the tumor. Vigorous microvesicle cavitation destroys tumor cells, effectively exposing tumor cells to a wide range of antigens, thereby promoting the maturation of APCs and subsequent adaptive immune cell activation [129]. In contrast, like other physical stimulations,

ultrasound also induces ICD [130]. Ultrasound-induced ablation directly destroys tumor tissues, as in HIFU therapy, and the ablation releases tumor-associated antigens and a variety of biologically active molecules, which release endogenous DAMPs (e.g., HSP-60 and ATP) to activate APCs. Activated APCs initiate T cells to for antigen-specific cellular immune responses [103]. During ultrasound ablation therapy, the increase in temperature also enhances blood perfusion and promotes circulation and penetration of immune cells in the target area [129]. Mechanical HIFU, in addition to increasing immune cell infiltration, facilitates the conversion of macrophages to an immunostimulatory M1 phenotype [131]. FU alone has immunostimulatory potential. Increasing the intensity of focused ultrasound in glioblastomas within safe limits was shown to increase tumor-infiltrating lymphocytes and produce an immunostimulatory TME [132]. Many studies have been conducted to show that ultrasound effectively assists immunotherapy. For example, Hu et al. [133] showed that the therapeutic effect of anti-PD1 monotherapy alone was weak when used to treat tumors but combined with ultrasound combined with nanobubbles (USNBs) significantly enhanced the effect of anti-tumor immunotherapy.

Conclusion and future perspectives

Various physical stimulations have intersections and differences in modulation of the TME, which mainly includes influencing the vasculature, ECM, and immune responses. The TME changes induced by physical stimulation not only facilitate the improvement of drug or nanomaterial delivery in resistant conditions but also modifies the immunosuppressive microenvironment, paving the way for immunotherapy. In general, physical stimulation can be used to modulate the TME as an adjuvant therapy and a stand-alone treatment for tumors. Treatment alone has the following advantages: 1) non-invasive or minimally invasive; 2) significant local therapeutic effect superior to chemotherapy and reduced side effects; 3) a curative role for early-stage tumors, achieving tumor reduction for middle- and late-stage tumors; 4) accurate positioning and good targeting; and 5) use as an adjuvant or combined therapy. However, physical stimulation, as a kind of pure auxiliary stimulation or therapeutic means, only targets stimulation to the local tumor site and can do nothing to the metastatic or spreading tumors. Combination therapy has brought new prospects. With the deep exploration of the TME and the advances of modern nanotechnology, targeted tumor treatment strategies using nanomaterials as carriers coupled with various physical stimulations have garnered widespread attention [66]. For example, the combination of physical stimulation with chemotherapy and immunotherapy shows complementary advantages, significantly improving tumor treatment efficacy and reducing the formation of metastatic lesions [134]. Additionally, therapies utilizing biological carriers, such as bacteria, combined with physical stimulation have also demonstrated promising

therapeutic effects [135]. In summary, this review has provided a reference for future research. As scientific inquiries delve further, combination therapy has become a focal point in current tumor treatment studies, creating favorable conditions for drug delivery and immunotherapy by modulating changes in the TME. Undoubtedly, combination therapy holds significant implications for future anti-tumor research. However, in the face of the complexity of the TME and the heterogeneity caused by individual differences, there is still a long way to go in exploring more precise and effective methods to modulate the TME and apply the findings in clinical tumor treatment. Despite the presence of numerous obstacles, we envision that the combination of physical stimuli-based cancer nanotherapy with chemotherapy drugs and immunotherapy will become an effective treatment approach in the near future.

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Declaration of interests

The authors declare that they have no competing interests.

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