Pulsed electromagnetic therapy in cancer treatment: Progress and outlook

Wenjun Xu¹,² | Xinxun Xie¹,² | Hanyang Wu¹,² | Xiaolin Wang³ | Jiancheng Cai¹,² | Zisheng Xu¹,² | Shiju E¹,²

¹Key Laboratory of Urban Rail Transit Intelligent Operation and Maintenance Technology & Equipment of Zhejiang Province, College of Engineering, Zhejiang Normal University, Jinhua, People's Republic of China
²Jinhua Intelligent Manufacturing Research Institute, Jinhua, People's Republic of China
³College of Mathematical Medicine, Zhejiang Normal University, Jinhua, People's Republic of China

Abstract
Cancer is one of the major diseases that endanger human health. Current treatment options have low patient tolerance, poor prognosis, and strong side effects. Pulsed electromagnetic therapy can selectively kill cancer cells by taking advantage of their own electromagnetic sensitivity specificity. Pulsed electric fields (PEFs), pulsed electromagnetic fields (PEMFs), and cold atmospheric plasma (CAP) are three main means that have promising application in cancer therapy. Pulsed electromagnetic therapy showed significant anticancer ability in vitro and in vivo. In this paper, we summarize the basic principles, methods, and state of art research of PEFs, PEMFs, and PEMFs for cancer therapy. Based on cancer pro-oxidation therapy, we propose a combination therapy of electromagnetic field-enhanced CAP for cancer therapy.

KEYWORDS
cancer therapy, cell experiment, cold atmospheric plasma, pulsed electric fields, pulsed electromagnetic fields

1 | INTRODUCTION
Cancer is a collective term for a range of related diseases. A typical feature of cancer is that the cells begin to divide without stopping and spread into the surrounding tissues. Many tumor cells form solid tumors. A solid tumor is similar to a separate tissue, which includes cancer cells, vascular tissue (including endothelial cells, perivascular cells, etc.), and stroma. The cells and components other than cancer cells in a solid tumor are not just the “microenvironment of cancer cells,” but they are interdependent with cancer cells." Currently, the incidence and mortality of cancer are increasing and have become the leading cause of death. The main therapies currently available for cancer include chemotherapy, radiotherapy, surgery, and a combination of these three means. The existing therapies cause
many side effects during treatment, are poorly tolerated by patients, and their survival cycles and quality have not been significantly improved.

Since this century, the research on bioelectromagnetics has gradually deepened. Its research contents include electromagnetic radiation protection, the study of the mechanism of electromagnetic field on organisms, and the electromagnetic biological effects. The electromagnetic-related therapy of cancer has become a research hotspot, including pulsed electric field (PEF), pulsed electromagnetic field (PEMF), and plasma therapy. Figure 1 shows the form of action of electromagnetic therapy for cancer. The PEF device (Figure 1A,B) is composed of a pulsed voltage source and an electrode. The voltage source generates a pulsed voltage, and an electric field is generated between the two electrodes. The PEMF device (Figure 1C,D) is composed of a current source and a magnetic field generating device. According to the principle of electromagnetic induction, a pulsed magnetic field is generated in the generating device for cells and tissues. Plasma is ionized gas from high voltage. The main components of plasma include electrons, ions, and neutral particles. Cold atmospheric plasma (CAP) (Figure 1E,F) has a temperature of $10^2$–$10^3$ K, and the pressure is close to atmospheric pressure. When CAP is applied to cancer cells or tissues, physical factors such as electromagnetic fields and ultraviolet rays, and chemical factors such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated. CAP has more abundant forms of action when it exposes on cancer cells, which can better avoid the drug resistance of cancer cells during treatment.

Among existing researches, PEFs have been widely used in biological and medical fields since 1980s. The experimental research on PEFs on cells has been relatively mature. Among them, tumor treating fields (TT Fields) initiated by Palti and coworkers of the Israel Institute of Technology, by interfering with the mitosis of tumor cells, realized the treatment of melanoma and glioblastoma cells. It has been approved by Conformite Europeenne (CE) and U.S. Food and Drug Administration (FDA) for the treatment of recurrent glioblastoma and locally advanced or metastatic malignant pleural mesothelioma.

Research on the biological effects of time-varying PEMFs has also been widely conducted. Most laboratory studies have focused on very low frequency bands, and clinical applications have mostly used pulsed or sinusoidal
magnetic fields below 1000 Hz with magnetic induction strengths within 100 Gs (1 Gs = 10⁻⁴ T). This pulsed magnetic field generally does not cause significant thermal effects in organisms due to the short duration of action and low irradiation power. The effects acting on organisms belong to the category of nonthermal effects. The organism itself is a medium with special characteristics of electromagnetic distribution. Changes in the external electromagnetic field can lead to changes in the trajectory of charged particles in the organism. It also affects the cell membrane potential, organelle membrane potential, etc. Such changes affect the function of membrane proteins, which can lead to changes in cell membrane permeability and cell membrane specific ions, ultimately affect the regulation of cellular functions. The magnetic permeability of the organism is close to that of μ₀ in vacuum, so the magnetic field has a strong penetration to the organism. 16

CAP has been increasingly used in cancer therapy. In in vitro experiments, CAP has shown effective killing effects on dozens of cancer cell lines, including brain, skin, breast, colorectal, lung, cervical, leukemia, liver, and head and neck cancers. In vivo experiments have shown that CAP irradiation is effective in reducing the size of tumors and improving the survival rate of subjects. 17

PEFs, PEMFs, and CAP are all transient actions, but there are some essential differences between them. PEFs and PEMFs are field therapies in which the treatment target is placed directly in the field, while direct plasma therapy is a kind of contact therapy in which the ionization-generated substance in question needs to be in direct contact with the treatment target. There are also studies of indirect therapy with ionization products for plasma, but they are not as effective as direct therapy.

2 RESEARCH STATUS OF PEFS CANCER THERAPY

2.1 Status of experimental research

The principle of PEFs acting on cancer cells is shown in Figure 2. PEFs are generated at the polar plates by loading voltage onto the two electrodes. When PEFs act on the cells, they trigger a series of cellular responses. Weaver 18 found that under the action of a PEF at a certain dose (field strength of 10³ V/cm level and pulse width of 10⁻⁶ s level), temporary hydrophilic channels called “microspores” will appear in the cell and organelle membranes. 19 At this time, the permeability of the cell membrane is greatly enhanced and the electrical conductivity increases greatly. After the PEF is turned off, in most cases, the microspores will close without any effect on the cell, and this physical process of temporary microspores in the cell membrane is called electroporation. When electroporation is produced, external ions such as Na⁺, K⁺ can enter the cell more easily. PEFs can cause cavitated, swollen mitochondria, loss of mitochondrial transmembrane potential, cytochrome c release, etc. When PEFs act on cell nucleus, double-strand break, H2AX fragmentation, disintegrated karyotheca will appear. PEFs can block the cell transfer from G0/G1 phase to S+G2/M phase. While the intracellular caspase can be activated, the Ca²⁺ can be released into cytoplasm, thus triggering apoptosis.

The electroporation effect is divided into reversible and irreversible electroporation. 20–22 When the electroporation effect is present, cells can better absorb various drugs, genetic material, proteins, etc. Studies have shown that the applied PEF dose triggers different degrees of membrane penetration. The number of pulses, pulse width, pulse steepness, voltage peak, and pulse frequency all can affect the membrane penetration efficiency. 23–25 PEFs need to have the following characteristics to be effective:

(i) The pulse rise time should be less than the charging time of the intracellular organelle membrane.
(ii) The pulse width should be greater than the charging time of the organelle membrane and less than the charging time of the cell membrane.
(iii) The electric field strength should enable the organelle membrane to complete charging during the pulse application. 26–29

From recent studies, the parameters can be seen from Table 1.

The effect of the PEF on the cells is therefore closely related to the time parameters. PEFs have a significant frequency window effect when applied to living organisms. When the PEFs frequency is below 1 kHz, the PEFs can excite tissues through membrane depolarization stimulation, thus stimulating bone growth and accelerating fracture healing. When PEFs frequency is higher than 1 MHz, the effect is mainly thermal, which can heat tissues, promote wound healing, and tumor cell ablation. When the PEF frequency is between 100 kHz and 1 MHz, with the increase of electric field direction change frequency, the effect of electric field force on charged ions and dipoles is nearly zero, which has no effect on quiescent cells. For mitotic cells, the inhomogeneous intracellular

|TABLE 1| Parameters of pulsed electric fields (PEFs) for reversible and irreversible electroporation |
|---|---|
| **Reversible electroporation** | **Irreversible electroporation** |
| Pulse width | Mostly 10⁻⁶ to 10⁻³ s | <10⁻⁵ s |
| Amplitude | Mostly 10⁵ to 10⁶ V/m | >10⁶ V/m |
FIGURE 2 The principle of pulsed electric fields (PEFs) acting on cancer cells

electric field generated by PEFs will disturb chromosome and cytoplasm separation.30

µPEFs have the characteristic of targeting to the cell membrane,31 enable successful plasmid DNA transfection, and induce exogenous proteins into the cell interior.32,33 The use of electrode needles or flat plate electrodes to deliver electric fields to the target area can lead to irreversible electroporation of the cell, resulting in the destruction of the cell membrane structure and necrosis of the cell.34,35 When a high-voltage pulse with width of 10–9 s levels and intensity of 10–100 kV/cm is applied, the abundant high-frequency component can target intracellular organelles through the shielding of the cell membrane, which can cause endogenous intracellular vesicles, contraction and swelling of cells, DNA damage to the nucleus, and cell necrosis or apoptosis.37 When PEFs are applied to cells, differences in the physical and electrical properties of the cells themselves will cause differences in inner and outer cell membranes electroporation effect.

nsPEF can induce perforation of the inner cell membrane and promotes the efficiency of gene delivery.38,39 Other studies have shown that conventional long pulses combined with nanosecond pulses can increase gene expression in the nucleus.40,41 Xiao and coworkers42 found that different pulse widths of PEMF induced apoptosis with very different effects: 10 kV/cm, 500 ns PEF induced very obviously apoptosis in human hepatocellular carcinoma HepG2 cells, 200 ns pulse also induced partial apoptosis, 100 ns pulse induced very low apoptosis, and 50 ns pulse stimulated cells were basically undetectable for apoptosis after 24 h.40,41

Garner et al.43 found that Jurkat cells exposed to five 10 ns, 150 kV/cm electrical pulses showed a dramatic decrease in cytoplasmic and nucleoplasmic conductivity after the pulses, corresponding to the previously observed increase in cell suspension conductivity. This indicates that electroporation occurred, resulting in ion transport from the inside to the outside of the cell. The delayed decrease in cell membrane conductivity after nsPEFs may indicate long-term ion channel damage or use-dependence due to repeated membrane charging and discharging.

Adachi et al.44 discovered that the human cervical cancer cells HeLa S3 cell death induced by 120 ns, 12.5 kV/cm pulses are dependent on the presence of Ca2+ and accompanied by expression of proteins associated with apoptosis and stress response. Conversely, the protein expression induced by 5 ns, 250 kV/cm pulses was independent of the presence of Ca2+. Nuccitelli et al.45,46 stated PEFs greater than 20 kV/cm with rise times of 30 ns and durations of 300 ns penetrate into the interior of tumor cells and cause tumor cell nuclei to rapidly shrink and tumor blood flow to stop. Melanomas shrink by 90% within 2 weeks following a cumulative field exposure time of 120 µs.37

2.2 Status of theoretical research

Theoretical studies of PEFs have focused on the simulation of cellular electroporation effects and the establishment of multiphysics field models. The electroporation progress study found that there was a significant cumulative effect on the distribution of perforation radius with the increase...
of the number of pulses. The radius of partial perforations on the outer cell membrane and the nuclear membrane gradually increased. The high-frequency nsPEFs with appropriate parameters can cause perforation of the cell nuclear membrane and play a role in expanding the radius of partial perforation of the cell.37,47–50

Further study simulated irreversible pore generation by introducing the effect of PEFs on the surface tension of the cell membrane and setting the irreversible pore radius threshold. The number of pulses and frequency all have a cumulative effect on the number of irreversible pores. The results of time-domain experiments on molecular transport confirm that cells have irreversible pores and that molecular transport increases with implosion frequency. When the relaxation time of the cellular transmembrane potential is longer than the delay between subsequent pulses, the presence of a threshold pulse repetition frequency is determined, which leads to the accumulation of charge on the membrane. This parameter can be used for flexible control of electroporation efficiency in the high-frequency range.51

In establishing a multiphysics field model of the cell membrane, the electric field is described by Poisson’s equation, ion transport by the Nernst–Planck equation (protons, hydroxides, sodium or calcium and chloride), mechanical equilibrium equations for the composition of the Maxwell tensor and membrane deformation with explicit discretization of the cell membrane, and membrane permeabilization by the Smoluchowski equation. Ezequiel Goldberg’s multiphysics field model describing electrical pulse–cell membrane interactions. It predicts that when PEF is applied to a spherical cell, its membrane will appear elastic deformation. Then the induced transmembrane potential, pore generation kinetics, and ion transport can be affected. In addition, consistency between maximum membrane deformation, maximum pore size, and maximum ion uptake is predicted.52–54 The nonlinear dispersive multiphysics field model for single-cell electroporation shows dispersive membranes would increase the transmembrane potential, accelerate the electroporation process, and weaken membrane permeability. However, adding dynamic pore radius functions had opposite effects on transmembrane potential and membrane permeability. The responses of cells exposed to unipolar and bipolar nanosecond pulse sequences were subsequently simulated.55 During the application of the monopolar pulse sequence, pore radius and perforation area accumulated in a stepwise manner, with a significant increase in perforation area and intracellular ion concentration with higher frequency of the pulse sequence and wider sub-pulse intervals. Bipolar elimination effects were also observed in terms of membrane permeability and pore radius.56

3 | RESEARCH STATUS OF PEMFS CANCER THERAPY

3.1 | Status of experimental research

PEMF cancer-related studies are mainly classified as inhibiting cell proliferation, directly killing or inducing apoptosis, altering cell cycle, affecting cellular pathways, etc. The principle of action is shown in Figure 3. The PEMFs is generally generated by the electromagnetic induction effect of current. A corresponding PEMF will be generated in the generating device. When PEMFs act on the cell, it will firstly change the cell membrane transport capacity, osmotic potential and ionic valves. Also, it will cause changes in mitochondrial protein profile, decrease mitochondrial phosphor-ERK (extracellular-signal-regulated kinase), p53, and cytochrome c, and activate OxPhos. In terms of the nucleus, PEMFs can repair DNA conformation, cause modulate gene expression, influence mitosis and chromosomal aberrations. The action of PEMFs decreases cellular stress factors, increase energy demand, this series of reactions will eventually lead to apoptosis.

We conclude parameters of the existing PEMFs research data as Figure 4 shows. The amplitude mainly focuses on lower intensity which is smaller than 100 mT. For frequency, the most common frequency used in the research is 50–60 Hz. About 26.1% studies used frequency in this range. The higher frequency is also a hotspot, about 23.9% studies focus on the range of larger than 1 MHz.

3.1.1 | Inhibition of tumor cell or tissue proliferation

Zeng et al.57 found that tumor growth in mice was inhibited by the effect of 0.6–2.0 T magnetic field. The metabolism, mitosis, and high-speed anomalous growth of tumor cells were inhibited in the irradiated group. de Seze et al.58 applied a magnetic field with an intensity of 0.12 T and a frequency of 0.8 Hz to natural killer cells and observed a significant increase in the proliferation of natural killer cells within 1 week compared to the previous one, indicating that a magnetic field of this intensity can improve the body’s ability to suppress cancer. Tuffet and de Seze found that 0.18 T, 0.8 Hz unipolar square wave pulsed magnetic field reduced the proliferation of isolated cervical cancer cells by 15%. Under the same parameters, pulsed magnetic field treatment for 8 h a day, 5 days a week, significantly prolonged the survival of tumor-bearing mice. The effect of the nonuniform field was better than that of the uniform field, with the pulsed magnetic field producing the highest induced current at the edge of the subject.
and the lowest induced current at the center. Cameron et al.\textsuperscript{59} used a pulsed magnetic field (15 mT, 120 pulses/s) to treat human breast cancer cells (MDA-MB-231) transplanted into mice. It was found that the growth of breast cancer was effectively inhibited in the irradiated mice compared to the nonmagnetic field irradiated mice, while the tumors in the original lung metastases in the mice were significantly reduced, with slow tumor growth and no significant harmful side effects. Filipovic et al.\textsuperscript{60} investigated the effects of a 50 Hz magnetic field on different types of cancer cells. Breast cancer cells MDA-MB-231, colorectal cancer cells SW-480, and HCT-116 were irradiated under a specific magnetic field for 24–72 h, and the results showed that the growth of all three types of cancer cells was significantly inhibited. Williams et al.\textsuperscript{61} further found that 10 min of daily 10, 15 or 20 mT pulsed magnetic field irradiation (120 pulses/s) significantly slowed tumor growth and reduced vascularization relative to mice not exposed to magnetic fields.

3.1.2 Induction of apoptosis

It has been shown that PEMF irradiation can reduce the proportion of polymerized microtubules by interfering with microtubule spindle polymerization. This disrupts the mitotic spindle structure and inhibits cell division, which leads to chromosome missegregation and induction of apoptosis in cancer cells.\textsuperscript{62} PEFs also have immunomodulatory effects, which can lead to increased levels of tumor necrosis factor (TNF) \(\alpha\), induce antitumor responses, and lead to activation of pro-apoptotic pathways induced by...
the interaction of caspase-8 with Fas.\textsuperscript{63} Yao et al.\textsuperscript{64} found that pulsed magnetic fields of 0.1–0.3 T had significant inhibitory effects on isolated murine myeloma cells, and the degree of cancer cell killing was related to parameters such as magnetic field amplitude $B$ and magnetic field change rate $dB/dt$. Redeva\textsuperscript{65} found that 50 Hz, 35 mT PEMF irradiation induced apoptosis in human lymphocytes cancer cells, but had no effect on normal lymphocytes cells. Wang et al.\textsuperscript{66} found that 1 mT, 8 Hz PEMF treatment significantly inhibited the proliferation of ovarian cancer cells (SKOV3) and induced apoptosis, studies have shown that the response of organisms to PEMFs is closely related to the parameters of the acting magnetic field.

3.1.3 Alters cell cycle, protein expression, and signal transduction pathways

Lee et al.\textsuperscript{67} found that very low frequency electromagnetic field action causes a delay in the cell cycle. Eleuteri et al.\textsuperscript{68} explored the effects of very low frequency electromagnetic fields on protein oxidation and 20 S proteasome function. Colon adenocarcinoma (Caco 2) cells were exposed to a 1 mT, 50 Hz electromagnetic field environment for 24–72 h. PEMF irradiation induced global activation of the catalytic component of the 20 S proteasome, which was particularly evident after 72 h of magnetic field irradiation. Many studies have also shown that PEMFs affect signal transduction pathways in cancer cells and alter ion binding and transport.\textsuperscript{69,70} Calcium ions are a key factor, and in a series of studies of calcium-calmodulin (CaM)-dependent myosin phosphorylation, it was demonstrated that specific PEMFs can modulate the binding of Ca$^{2+}$/CaM, thereby enabling Ca$^{2+}$ in cell-free enzymatic preparations. Twofold enhanced binding kinetics.\textsuperscript{71}

PEMFs was used to enhance gene delivery. It significantly increased the transfection efficiency by a factor of 40. When the nanoparticles were deposited on the permanent magnet before the pulsed field was applied, the 50 nm and 200–250 nm nanoparticles had the highest transfection efficiency.\textsuperscript{72} Combination treatment with PEMFs and the antineoplastic drug mitomycin C (MMC) showed that 90-day survival rates is 34% in the MMC-only group. In the PEMFs-only group, the rate is 47%. The combined group's rate was 77%, indicating that the addition of PEMFs can improve the efficacy of drug therapy. This combination experiment provided new ideas for possible alternative treatments for cancer.\textsuperscript{73} PEMFs will control the cell growth according to the degree of cell differentiation. When ADR-resistant osteosarcoma cells were exposed to PEMFs, the growth rate of resistant cells was significantly lower than unexposed resistant cells. At the same time, studies also showed that PEMFs can promote the growth of undifferentiated cells, while inhibit the cell with higher differentiation.\textsuperscript{74}

3.2 Status of theoretical research

Scholars have studied the mechanisms of interaction between weak electric and magnetic fields and biological systems. Many hypotheses and mechanisms have been proposed and explored so far, such as ion cyclotron resonance, ion parameter resonance, free radical concept, heat shock proteins, etc. The originally proposed model uses a linear physicochemical approach\textsuperscript{75} to build an electrochemical model of the cell membrane to evaluate the electromagnetic field parameters. It is hypothesized that nonthermal electromagnetic fields can directly affect ion binding and transport and may alter biological processes related to tissue growth and repair. This electrochemical information transfer hypothesis postulates that the interaction between the cell membrane and electromagnetic fields can regulate the rate of ion binding to receptor sites. Several different types of electrochemical interactions may occur at the cell surface, including nonspecific electrostatic interactions. At the interface between the water dipole and the lipid bilayer of the cell membrane, hydrated and voltage-dependent ions bind to each other.\textsuperscript{76}

Liboff et al. proposed the ion cyclotron resonance (ICR) hypothesis based on the idea that calcium ions would cyclotron resonate in the presence of a magnetic field. The condition of resonance is that when the frequency of the alternating magnetic field is close to the Larmor frequency of the DC magnetic field-ion system, the magnetic field of specific parameters can increase the mobility of specific ions near the acceptor site and through the ion channel.\textsuperscript{77} The ICR model cannot explain the lack of bare ions caused by ion hydration in the organism, the actual measured ion migration velocity is much lower than the calculated value, and thermal noise.\textsuperscript{78}

To address the thermal noise problem in the ICR model, Lednev\textsuperscript{79} proposed the ion parametric resonance (IPR) model, in which ions in macromolecular binding sites are modeled as charged harmonic oscillators.\textsuperscript{80,81} The IPR theoretical model requires that the wells where the ions are located must be spherical or tetrahedral, and a small deviation from the symmetry will cause huge deviations in theoretical predictions. Liboff et al. demonstrated that the activity of CaM-dependent cyclic nucleotide phosphodiesterase was significantly changed under the action of a 20 $\mu$T magnetic field, and the induced electric field generated by the PEMFs was much lower than the thermal noise threshold, but a significant biological effect is produced,\textsuperscript{77} so the thermal noise problem when the PEMFs is applied can be ignored.
Taking thermal noise into account, Larmor precession (LP) model was proposed by using a classical mechanical approach to the problem of ion motion under the action of a magnetic field.\textsuperscript{82,83} The calculation results of this model show that thermal noise can transfer the magnetic field energy to the protein through ions, thereby triggering the biological effect. The LP theory describes the effect of magnetic fields on ion binding kinetics. It has been suggested as a possible mechanism for the observed biological effects due to weak exposure to electrostatic and alternating magnetic fields.

Many animal studies as well as clinical experience have shown that the initial conditions of the target of PEMP action determine whether physiologically meaningful biological effects can be achieved. For example, when fractures are treated with PEMFs, the surrounding soft tissues receive the same dose as the fracture site, but physiologically significant changes occur only in the damaged bone tissue, and no changes are observed in the surrounding soft tissues. Studies on Jurkat cells found that lymphocytes after receiving other stimuli responded more significantly to magnetic fields than normal T lymphocytes. Based on such experimental phenomena, Hazlewood and Markov\textsuperscript{84} proposed the pendulum effect of magnetic fields acting on organisms: the greater the deviation from equilibrium state, the stronger the response to magnetic field action.

### 4 RESEARCH STATUS OF CAP CANCER THERAPY

The principle of CAP acts on cancer cells as shown in Figure 5. CAP is a mixture of various reactive oxygen, reactive nitrogen, charged particles, ultraviolet light, etc. The high-energy electron bombardment of the solution generated by CAP during irradiation can ionize water molecules and excite the generation of free oxygen, ozone, free radicals, and other highly reactive substances, which can better kill bacteria, viruses, cells, and other living organisms by the synergistic effect of physical factors such as electromagnetic field and ultraviolet light when combined with irradiation. The application of CAP in cancer therapy is a typical ROS-pro-oxidant therapy. CAP-treated media will contain many long-lived harmful chemicals originating from CAP, including ROS such as H$_2$O$_2$, and RNS such as NO$^2$-, NO$_3^-$, and NO. When CAP acts on cancer cells, it will increase the intracellular ROS level. CAP can cause mitochondrion damage, loss of mitochondrial transmembrane potential, p53 phosphorylation, and activate Bax, and PUMA and NOXA (pro-apoptotic factors). Also CAP will damage DNA, break DNA double-strand, cause ataxia telangiectasia mutated andy-H2AX's serine 139 phosphorylation, etc.

Depending on the form of plasma action with the treated object, it can be divided into direct and indirect plasma action.\textsuperscript{36} Direct plasma action means that plasma acts directly on the irradiated object, including cancer cells, tumors, etc. Indirect plasma action involves irradiating a medium such as water or culture medium with plasma, generating active particles such as ROS and RNS in it, and then culturing the cells or tumor tissue with the irradiated medium. The research of plasma in cancer therapy mainly includes inhibition of tumor cell proliferation and induction of apoptosis, inhibition of tumor angiogenesis and migration, combined therapy with drug, and study of ROS/RNS generation mechanism.

#### 4.1 Status of experimental research

**4.1.1 Inhibition of cell proliferation and induction of apoptosis**

CAP can inhibit cell growth and migration, induce apoptosis, trigger cell cycle arrest and cancer cell senescence, and mitochondrial dysfunction.\textsuperscript{85} Iseki et al.\textsuperscript{86} treated two independent ovarian cancer cell lines with plasma and found that plasma treatment significantly reduced the proliferation rate of ovarian cancer cells, induced apoptosis in ovarian cancer cells. CAP action on cells can induce apoptosis in melanoma cells by upregulating intracellular ROS levels and inducing apoptosis.\textsuperscript{87} In studies of CAP-treated melanoma cells, the TNF receptor (TNFR)-based apoptotic pathway has been activated by increased intracellular ROS levels. Most of the apoptotic pathways observed in CAP-treated cancer cells are based on DNA damage and mitochondrial damage, and plasma was found to inhibit head and neck cancer (HNC) tumor growth and lead to increased levels of intracellular ROS in a nude mouse xenograft model in CAP-treated HNC studies, and CAP can simultaneously induce apoptosis in HNC cells by modulating ROS levels in mitochondria.\textsuperscript{88} Kim et al.\textsuperscript{89} investigated the proliferation and death phenomena of rectal cancer cells in the presence of CAP and found that plasma treatment increased the phosphorylation of $\beta$-linked proteins, which demonstrated that plasma could be used as a novel modifiable tool for cell signaling and function with therapeutic potential. In plasma-induced apoptosis experiments in cancer cells, it was also found that plasma can lead to sub-G1, G2/M cell cycle cell block in cancer cells.\textsuperscript{90} In plasma-induced apoptosis experiments in oral squamous cell carcinoma (OSCC), sub-G1 cell cycle block was found in normal OSCC but not in p53-mutated OSCC, that is, plasma-induced apoptosis and sub-G1 phase block were associated with DNA damage and ATM/p53 signaling pathway in cells.\textsuperscript{91} Similar results were
obtained for CAP treatment of all types of tumor tissues in experiments with ruffled mice, where plasma treatment slowed the growth tendency of tumor cells, reduced the size of tumor tissue, inhibited the growth of tumor tissue, and improved the survival rate of mice.92,93 Plasma treatment experiments in mice implanted subcutaneously with bladder cancer confirmed a significant reduction in tumor volume after 24 h of 2-min plasma treatment.94 In a study on mice with melanoma, CAP achieved a cure rate of 66.6% for melanoma.95

4.1.2 Inhibit the spread and migration of tumor cells

The spread migration of tumor cells and invasion of surrounding tissues is a major feature of cancer, therefore, effective inhibition of tumor cell migration is an important topic in cancer therapy, and the treatment of tumor metastasis is also of great importance in the field of cancer medicine. Existing studies have confirmed that plasma can significantly inhibit the migration and proliferation of cancer cells.96,97 Plasma can reduce tumor cell motility and colony formation, and transcriptome-wide genetic analysis has shown that plasma treatment is associated with inhibition of migration and disintegration of the actin cytoskeleton mediated through multiple signaling pathways.98 Kim et al.99 used helium and a mixture of helium and oxygen as carrier gases, respectively, to treat colorectal cancer cells with plasma jets, found that as the concentration of plasma and oxygen added to helium increased, the invasion and metastasis of colorectal cancer cells were reduced. Chang et al.100 found that plasma inhibited the invasion and metastasis of human papillary thyroid cancer cells (BHP10-9) by decreasing matrix metalloproteinase-2/-9 and urokinase-type fibrinogen activator (uPA) activities and rearranging the cytoskeleton cancer cells (BHP10-3 and TPC1) invasion and metastasis, and these findings suggest that CAP may be used as part of a new therapeutic strategy to inhibit invasive and metastatic cancers.

4.1.3 Combination therapy with drug

The formed analysis shows that one of the major problems in cancer drug therapy is that cancer cells tend to develop resistance to drugs, while drug concentrations sufficient to kill cancer cells also tend to produce large side effects on normal cells and tissues. In addition to directly killing cancer cells and inducing apoptosis, plasma can also be used in conjunction with anticancer drug therapy to increase the sensitivity of cancer cells to drugs. In studies of colorectal cancer cells, plasma can selectively induce colorectal cancer cell death by activating the TNF and cysteine 3/7 apoptotic pathways, while increasing the sensitivity of apoptosis-inducing ligand (TRAIL)-resistant colorectal cancer cells to TRAIL.101 In studies of glioma cells, irradiation with plasma restored the sensitivity of temozolomide (TMZ)-resistant glioblastoma cells to TMZ, and co-treatment with plasma and TMZ resulted in cell proliferation inhibition.
and cell cycle arrest. Kim et al. used plasma to treat melanoma cancer cells that had been pretreated with gold nanoparticles fused with anti-FAK antibodies, and this surface-modified nanoparticle weakened the normal function of focal adhesion kinase (FAK), thus promoting melanoma cell detachment from the stroma detachment. Lee et al. increased cancer cell death and reduced epithelial-mesenchymal transition (EMT) in solid tumors using a combination of polyethylene glycol-coated gold nanoparticles and plasma treatment. In addition to the use of nanoparticles, drug-encapsulated core–shell nanoparticles synthesized by coaxial electrospray have shown their synergistic anticancer potential with plasma on breast cancer cells. These studies suggest that nanoparticles may impair or disrupt the normal function of specific proteins or pathways whose changes are caused by active CAP substances.

4.1.4 Study on the mechanism of ROS/RNS generation

The interaction between plasma and the tissues, cells and culture medium grown in the culture dish forms the basis of the anticancer effect of plasma. During plasma action, many active substances are produced, among which oxy species such as hydroxyl (OH), singlet oxygen (O2), superoxide (O2−), hydrogen peroxide (H2O2), ozone (O3), and nitrogen-based species such as nitric oxide (NO), nitrogen dioxide (NO2), nitric oxide (NO), nitrous oxide (N2O) and dinitrogen tetroxide (N2O4) are observed in the CAP. CAP also produced positively charged ions such as N2+ and electrons during discharging. Plasma-derived ROS and RNS are considered to be the main factors of cell death due to CAP irradiation, current studies suggest that apoptosis of plasma-treated cancer cells is mainly due to DNA double-strand breaks caused by a significant elevation of intracellular ROS. Yan et al. applied plasma jets to liver cancer cells (HepG2), the results showed that plasma could effectively control the intracellular concentrations of ROS, NO, and lipid peroxides, which were directly related to the mechanism of HepG2 death. The researchers thus hypothesized that the mechanism of plasma action on cancer cells is divided into the following steps: Firstly, plasma produces NO and other substances leading to an increase in the concentration of NO in the extracellular medium. Secondly, the intracellular NO concentration increases due to NO diffusion from the medium. Third, the increase in intracellular NO concentration leads to an intracellular stress response, resulting in an increase in intracellular ROS concentration. Fourth, the increased oxidative stress leads to more efficient lipid peroxidation and consequently to cellular damage. The combined effect of NO, ROS and lipid peroxides substances eventually leads to HepG2 cell death.

The research of PEF for cancer treatment is based on the electroporation effect of cells, which can effectively induce apoptosis of tumor cells, induce anti-vascular and lymphatic metastasis effect and immune effect of the body, destroy the survival condition of tumor, and reduce the metastasis of tumor cells. The number and effect of electroporation can be adjusted by regulating the parameters of the PEF, which can be combined with different drug treatments to improve the efficacy of existing therapies. Also, it has been applied clinically as a primary therapy. In the application, the efficacy of PEF still needs to be improved, and electric field as a contact therapy for cancer in vivo still needs to be enhanced.

In existing studies, PEFs can cause ablation of tumor tissues when they are applied to tumor tissues, however, the selection of pulse parameters and electrode arrangement of PEFs are mostly determined empirically. For different cancer cells, due to their different geometric parameters, physiological structure, and electrical characteristics, the corresponding targeting PEFs are different. To achieve precise targeting therapy of PEFs, it is necessary to measure the characteristic parameters of cancer cells, establish an accurate cell electrical model, and design the relevant parameters of PEFs according to the calculation results of the time–frequency characteristics of the cell model. In the experiment, the correspondence between each pulse parameter and the inhibition effect of cancer cells should be established to realize the precise regulation in the treatment plan, and the dynamic adjustment of the treatment...
plan can be realized by combining the previous model calculation and the experimental results.

Existing research on pulsed magnetic fields focuses on the study of biological effects of very low frequency pulsed magnetic fields. When pulsed magnetic fields act on tumor cells or tissues, they can inhibit the formation of microvasculature in tumor tissues, cause edema in mitochondria and rough endoplasmic reticulum of tumor cells, improve the anticancer ability of human immune cells, and change the transmembrane potential of cell membranes and the concentration of calcium ions in membranes.\textsuperscript{112–114} The current research on PEMF cancer therapy is mainly divided into experimental studies and mechanistic studies of the biological effects of the action of pulsed electromagnetic environment based on the results of experimental studies. Among them, the results of their biological experiments are mainly classified as inhibiting cell proliferation, directly killing or inducing apoptosis, altering cell cycle, and affecting cellular pathways. The existing studies on the biological effects of PEMFs on tumors are still limited to the measurement of a single waveform, a single parameter, and a single time point. The organism is a continuously changing system with strong self-regulation function. It has its own regulatory mechanism to respond to the external world, rather than a simple linear response. Therefore, a more systematic and coherent study is necessary to gain a deeper understanding of the biological effects of PEMFs on tumors. When designing the magnetic field form, parameters such as the rising edge, duration, and action integral should be fully considered, and multiple magnetic field parameters should be considered for the experiment, and multiple time points should be sampled, so that the whole process of the physiological response of the irradiated living organism to the magnetic field can be recorded more completely.

Plasma application for cancer therapy is a very emerging field, and so far, current research has focused on the intuitive treatment of cancer cells as well as tumor tissues by plasma, with very limited understanding of the anticancer mechanism. Studies have shown that the main contribution of plasma to cell killing originates from H\textsubscript{2}O\textsubscript{2} in the culture medium, and that plasma acting on cells or living tissues is a complex external effect integrating multiple physical and chemical effects. Existing studies tend to ignore the physiological changes caused by plasma acting on living bodies and focus on the subsequent damaging effects caused by the active particles brought by plasma, which leads to inability to explain the difference between plasma and H\textsubscript{2}O\textsubscript{2} with the same concentration. To become a mature anticancer treatment modality with a solid theoretical foundation, the underlying mechanisms need to be addressed. In addition to the effect of short-lived active substance concentration, the direct action of plasma causes changes in cellular stress response and state that are also worth exploring.

CAP is a typical ionizing radiation, which is a combination of pulsed electromagnetic environment and active particles such as ROS. So far, studies have mainly focused on the intuitive treatment of cancer cells and tumor tissues by plasma, and the understanding of the anticancer mechanism is very limited, and the response logic of each part of the organelle is not clear. The effect of CAP on the selective killing of cancer cells depends mainly on the ROS and electromagnetic sensitivity specificity of different cells, while the effect of CAP on the electromagnetic environment is transient. To enhance the efficiency of CAP action and precise treatment, it is necessary to improve the permeation efficiency of ROS and selectively open the ion channels of cell membrane by PEFs or PEMFs to reduce the impact on normal cells.

To improve the effectiveness and reduce the side effects of current cancer therapies, the differences between cancer cells and normal cells should be distinguished in order to achieve targeted cancer therapy. In the normal physiological process of cells, cancer cells are in a higher redox state compared to normal cells,\textsuperscript{115} and this high level of ROS is important for the growth and proliferation of tumor cells, infiltration and metastasis, and chemotherapy resistance,\textsuperscript{116} while excessive ROS can cause oxidative stress, damage to DNA, oxidized lipids and proteins, etc., leading to apoptosis and necrosis.\textsuperscript{117,118} Low concentrations of ROS promote cell division, medium concentrations of ROS lead to cell growth arrest, and high concentrations of ROS lead to apoptosis or cell death.\textsuperscript{119} The types of ROS include superoxide anion (O\textsuperscript{2–}), hydroxyl radicals (OH\textsuperscript{–}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), peroxyxinitrite, etc. The various types of ROS have different regulatory roles in cell growth. O\textsuperscript{2–} catalyzing the oxidative breakdown of lipids and forming metabolites that induce DNA mutations and attack cell membranes, and H\textsubscript{2}O\textsubscript{2} regulating apoptotic pathways such as JNK and P38/MAPK pathways to induce apoptosis.\textsuperscript{120}

The intracellular ROS content of cancer cells is much higher than that of normal cells, and therefore cancer cells are less tolerant to external ROS than normal cells. The approach of treating tumors by regulating the redox status of cancer cells and using the antitumor properties of ROS to achieve therapeutic goals by elevating the level of ROS inside cancer cells is called a pro-oxidant regimen for cancer therapy.\textsuperscript{121,122} In this regimen, research focuses on effectively enhancing the efficacy of ROS, reducing cancer cell resistance, and reducing the impact on normal tissues to achieve targeted and accurate treatment of cancer cells.

Cancer cells differ significantly from normal cells in terms of physiological, biochemical, and morphological characteristics. Cancer cells are usually larger in size, contain significantly more water, have a higher nucleoplasmic...
ratio than normal cells (up to 1:1). Cancer cells have a variable nuclear morphology, and may appear meganucleated, binucleated, or multinucleated. Therefore, there are significant differences between the structure and various dielectric parameters of cancer cells and normal cells. When subjected to applied electromagnetic field treatment, the two types of cells exhibit distinct responses, that is, the electromagnetic sensitivity specificity of cancer cells. Under normal conditions, the cell membrane is a permeable barrier between the cell and the outside world, and its basic function is to maintain the relative stability of the intracellular microenvironment and to selectively exchange substances with the external environment. When electroporation appears, the permeability of the cell membrane is greatly enhanced. Some large molecules, such as drug molecules, genetic material, and proteins, can enter the cell and be absorbed by the cell smoothly. Due to the differences in differentiation and structural characteristics between cancer cells and normal cells, the critical transmembrane potential and electric field triggering conditions required for the electroporation effect are different. The nucleoplasmic ratio and the dielectric constant of the cell membrane have a greater influence on the charging characteristics of the cell nuclear membrane under the action of PEF, and the internal electric effect is more obvious in cancer cells than in normal cells. Taking advantage of this feature, targeted therapy against cancer cells can be better achieved. In the pro-oxidation scheme of ROS, the electroporation effect of PEF can be used to open the channel of exogenous ROS into cancer cells and improve the penetration efficiency of ROS. The scheme is shown in Figure 6. The electromagnetic response difference between cancer cells and normal cells can be used to reduce the effect of ROS on the intracellular organelles of normal cells. The target selection is based on the electroporation level and ROS tolerance level to effectively treat cancer without affecting normal tissues.

The source and concentration of ROS are also critical in ROS pro-oxidant therapy regimens for cancer, as the effect of a single ROS tends to make cancer cells resistant and reduce the efficacy of the therapy. CAP is a very emerging field in cancer therapy and is a typical ROS pro-oxidant therapy. CAP-treated medium contains many long-lived harmful chemicals originating from CAP, including ROS and RNS such as $\text{H}_2\text{O}_2$, $\text{NO}^2^-$, $\text{NO}^3^-$, and NO. The abundance of ROS originating from CAP can effectively prevent cancer cells from developing drug resistance, and studies have shown that CAP irradiated solutions containing equal concentrations of ROS irradiated solution is better than single $\text{H}_2\text{O}_2$ solution in inhibiting cancer cells. The damage to normal tissues cannot be avoided in conventional cancer therapy. The targeted therapies from the perspective of electromagnetic and ROS content differences between cancer cells and normal cells, can greatly improve the efficiency and reduce the impact on healthy tissues. The introduction of PEFs and PEMFs can improve the penetration efficiency of ROS, not only reduce the concentration of drugs, but also reduce the irradiation dose of CAP, which can be applied to cancer treatment.
and provide an experimental basis for the combination therapy with existing therapies.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

ORCID
Zisheng Xu https://orcid.org/0000-0002-0411-0325

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AUTHOR BIOGRAPHIES

Wenjun Xu received his PhD degree in Electrical Engineering from National Laboratory of Power Equipment and Electrical Insulation, Xian Jiaotong University, China. She is currently a lecturer with the College of Engineering, Zhejiang Normal University, China. Her research interest is pulsed electromagnetic field bioeffects.

Zisheng Xu received his PhD degree in Electronics Science and Technology from Wuhan National Laboratory for Optoelectronics (WNLO), Huazhong University of Science and Technology (HUST), China, in 2020. He is currently a lecturer with the College of Engineering, Zhejiang Normal University, China. His research interest is energy harvester, biomedical electronics, and self-power wearable sensor.

Xinjun Xie received his BS degree from Zhejiang Normal University in 2020. He is currently a joint training MD student in College of Engineering, Zhejiang Normal University, under the guidance of Professor Shiju E. and Zisheng Xu. His research interest is energy harvester, biomedical electronics, and self-power wearable sensor.

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