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Review

Tumor treating fields for high-grade gliomas

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ABSTRACT

High-grade gliomas (HGG) are the most common malignant intracranial tumors with poor prognosis. Current treatments have not yielded optimal remission rates; there are no standard treatments for recurrent and drug-resistant gliomas. Tumor treating fields, which was recently approved by the Food and Drug Administration (FDA), could significantly improve progression free survival and the overall survival of glioma patients. In this review, we elaborate on the mechanism of tumor treating fields in tumor cells and detail various preclinical and clinical studies on gliomas. Tumor treating fields could be a promising option for patients with malignant tumors for which there are no standard treatment plans. Moreover, we identify several potential problems for the practical application of tumor treating fields and predict future directions for further studies. Tumor treating fields may be a potential therapy with high efficacy, fewer adverse effects, and high cost-effectiveness.

1. Introduction

Gliomas originate from glial cells and are the most common intracranial tumor. According to the World Health Organization (WHO), grade III and grade IV gliomas belong to high-grade gliomas (HGG), whose morbidity is (3-5)/100,000 and preferentially affects 50-60 years old men [1]. Glioblastoma multiforme (GBM) accounts for 46% of primary brain cancers and has a high incidence, recurrence rate, and mortality and a low cure rate with an overall survival (OS) of approximately 14 months [2].

In the past 10 years, little progress has been made in the treatment of HGG [3]. The identification of several biomarkers is promising for predicting the prognoses of glioma patients [4,5]. However, based on current treatment methods, the prognostic prediction of high-grade gliomas remains frustrating and typically results in relapses after surgery and standard radiotherapy and chemotherapy. At present, no universally acknowledged standard treatment plan exists for recurrent HGG. Some scholars believe that tumor treating fields (TTFields) can significantly improve progression free survival (PFS) and OS [6]. In this review, we elaborate on the mechanisms of electric field therapy and review preclinical and clinical studies to evaluate the efficacy of TTFields in HGG and other malignant tumors. Moreover, we discuss potential issues during clinical practice and future directions in the field. TTFields can be a promising approach in HGG treatment.

2. Mechanism of TTFields

2.1. TTFields in cell electrophysiological activities

Cell electrophysiological activities play important roles in many physiological processes, such as signal transduction and mitosis [7]. Biological cells contain many polar and charged molecules and ions, such as proteins and DNA. Under the influence of an alternating electric field, these molecules and ions will move in a certain direction. In a uniform electric field, the direction of the electric field force is parallel to the direction of the electric field (Fig. 1) [8]. In an inhomogeneous

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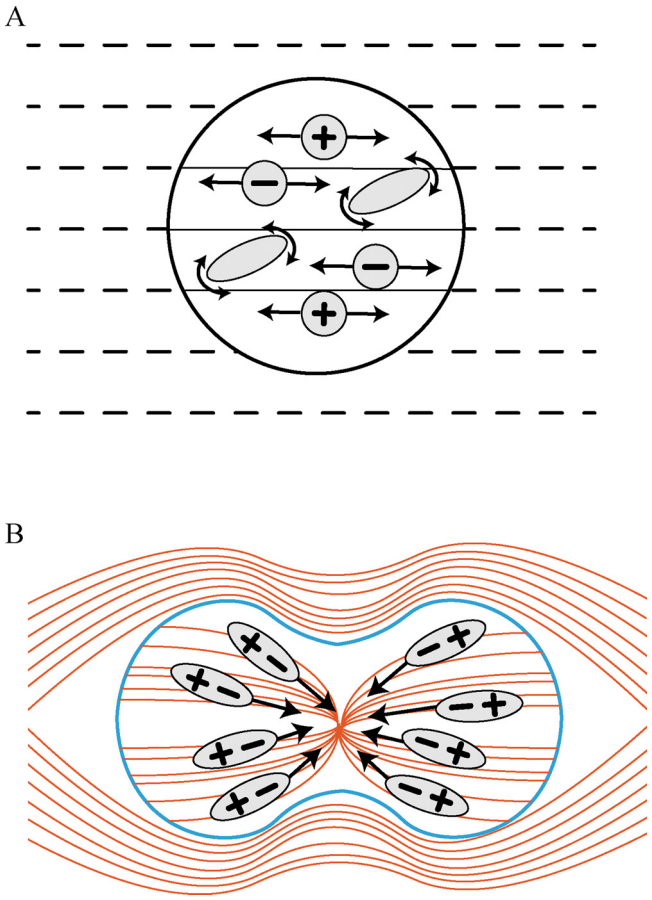

Fig. 1. AC field distribution in and around quiescent and dividing cells. **A**. Inside quiescent cells, the field is uniform, and the oscillating electric forces result only in the "vibration" of ions and dipoles. **B**. In contrast, the highest electric field density is in the center of the stenosis, which induces forces that push all dipoles toward the furrow. Adapted from Kirson, E.D., et al. (10). Copyright (2007) National Academy of Sciences.

electric field, all charged molecules will move toward the direction of high field strength. When the frequency of the electric field is sufficiently high, for example, in the case of tumor treatment electric fields, the intensity of the molecular movement is weakened. The electric field in nondividing cells is usually uniform, and the electric force often causes slight movements of electric charges and dipoles. However, electric field forces generated by a non-uniform electric field will push charged molecules to move toward the direction of high field strength, i.e., two-dimensional electrophoresis [8]. The inhomogeneous electric field is unique to cell division.

2.2. TTFields interfere with mitotic spindle formation

Mitosis is known as indirect division. During mitosis, the spindle distributes duplicated chromosomes equally to two daughter cells. Microtubulin, a tiny polar molecule, polymerizes into bar subunits, extends to the genetic material arranged at the cell center, and binds to chromosomes [9]. In TTFields, the microtubule subunits in cells without mitosis are arranged in parallel according to the direction of the electric field [10]. Finite element analysis shows that TTFields selectively affects cell division, whereas nondividing cells remain undisturbed. TTFields disrupts microtubules to impede mitosis and spindle formation. Due to interactions between the inherent dipole in mitosis, the microtubule protein dimer is parallel with the direction of the applied electric field and not with the microtubule axis; this prevents microtubule formation and affects spindle formation [11]. Septin 7 has been reported to be sensitive to treatment with TTFields in HGG patients (Fig. 2) [12]. Mitosis abnormally halts, and cell division stops. TTFields can also induce tumor cell mitosis to stagnate in interphase (G1/S/G2) for a prolonged period, preventing the mitosis cycle of tumor cells and even causing cell fragmentation [10]. These abnormal mitotic events induced by TTFields lead to abnormal chromosome separation, multinucleation, and apoptosis.

2.3. Disorder of the cell structure caused by dielectrophoresis

In the middle and later stages of mitosis, the cell membrane begins to constrict, and the two sets of chromosomes are pulled to the two poles of the cell. A cleavage ditch is subsequently formed, and the two daughter cells are completely separated. The mitotic groove is a narrow membrane junction. TTFields form an hourglass-like nonuniform electric field and a nonconstant electric field at this narrow cell membrane junction. The highest electric field density is located in the center of the stenosis as shown in Fig. 1 [10]. Polar molecules and cell dipoles are affected by strong electric field forces and move to the cleavage ditch, which is a process termed dielectrophoresis, leading to structural disorder and dysfunction in the cell and eventually leading to apoptosis [10]. Thus, TTFields can induce dielectrophoresis in cells in the middle and late stages of mitosis, which leads to the disorder and destruction of tumor cell structure and ultimately inhibits tumor growth (Fig. 3).

2.4. Expression of different cell states under TTFields

Different cell states have different manifestations under TTFields [12]. TTFields act on static cells, and the electric field is uniform. Oscillating electric field forces induce vibrations in ions and dipoles. However, the nonuniform electric fields formed by TTFields in mitotic cells induce all dipoles to advance toward the cleavage ditch. TTFields applies a directional force, impeding spindle formation. The stop or delay of mitosis may be caused by the improper attachment of chromosomes to spindle fibers. Cells die during mitotic arrest or cell division, leading to abnormal aneuploidy. Abnormal daughter cells die in the subsequent intermediate stage and are permanently arrested or blocked by TTFields again.

3. Preclinical studies on TTFields

3.1. Cell experiments

A series of in vitro studies reported that TTFields inhibited proliferation and killed tumor cells, including melanoma, glioma, and lung, prostate, and breast cancer cells. These studies observed that the electric field frequencies capable of inhibiting proliferation are dependent on the sizes and shapes of cells [8]. The optimal inhibition frequency of B16F1 cells was 100 kHz, that of human breast cancer cells was 150 kHz and that of F98 cells was 200 kHz. The effect of TTFields on inhibiting tumor cell division and promoting apoptosis was found to be related to the electric field intensity. In a given range, the inhibitory effect on tumor cells gradually increases with increasing electric field intensity [13]. Different cells have different sensitivities to electric field intensities. The most sensitive cells are rat melanoma cells, and the least sensitive cells are human breast cancer cells [13]. The effect of TTFields is related to the direction of the electric field and the splitting axis. The inhibition effect is greatest when the two directions are parallel; the effect is weakest when the two directions are vertical. In culture, the axis direction of cell division is randomly arranged, and only a few mitotic cells are exposed to ideal treatment. By alternating the electric fields in multiple directions, the effect of two mutually perpendicular electric fields was found to be 20% higher than that of a field with a single direction [8,13,14].

The duration of mitosis of tumor cells is prolonged or even damaged when observed under microscope. Under the intervention of TTFields,

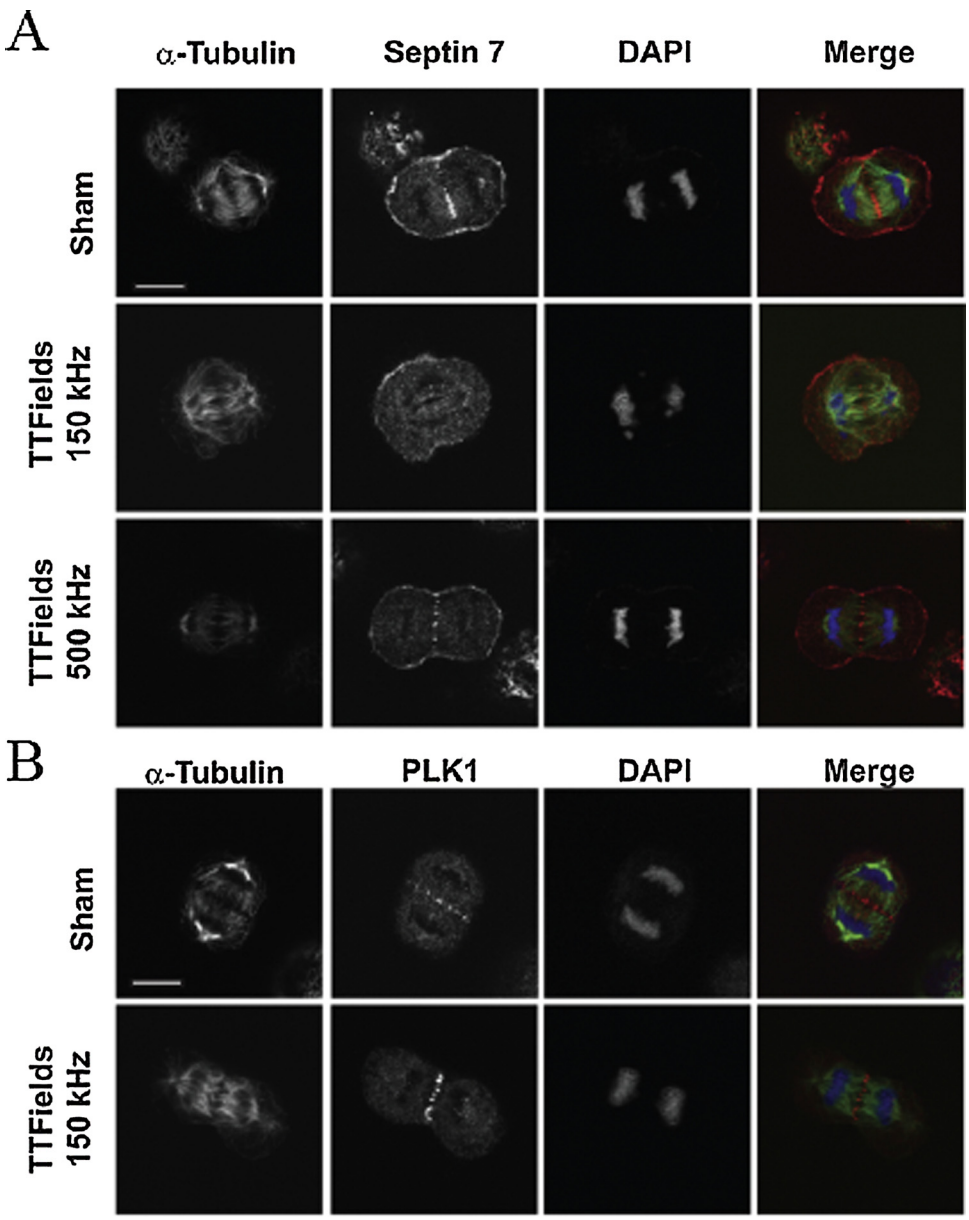

Fig. 2. Septin 7 localization is perturbed by TTFields. Synchronized cells were cultured in the absence of TTFields or in the presence of TTFields at 150 kHz (n = 30) or 500 kHz (n = 12), fixed and stained with antibodies against α-tubulin and Septin 7 and counterstained with DAPI. Confocal microscopy of cells captured in mid-anaphase under these conditions revealed that Septin 7 localization was reduced and the midlines of the anaphase spindles were perturbed by TTFields at 150 kHz and to a lesser extent at TTFields at 500 kHz compared to those in control cells (n = 27) (**A**). In contrast to that of Septin 7, PLK1 localization to the anaphase spindle midline appeared unperturbed by TTFields at 150 kHz (n = 11) (**B**). Adapted from Gera, N., et al. (12).

immunohistochemical staining showed tumor cells undergoing abnormal mitosis, which is due to intervention by TTFields in spindle formation. These abnormalities were similar to those observed when paclitaxel and other chemotherapy drugs interfered with tumor cell proliferation. Other studies have shown that TTFields combined with chemotherapy drugs had improved efficacy [15]. TTFields align cells in the direction of the electric field. The electric force is greatest when the mitotic axis is in the same direction as the electric field. The position has also been suggested to affect sensitivity to TTFields.

3.2. Animal experiments

Animal experiments using TTFields showed that an alternating electric field with a moderate frequency had an inhibitory effect on tumor proliferation (Fig. 4) (the study was approved by the Institutional Review Board; the animal use complied with the Guide for the Care and Use of Laboratory Animals) [8]. In the experiment, 12 pairs of malignant melanomas implanted subcutaneously in mice were treated by TTFields using an inner electrode. Similarly, 12 pairs of gliomas implanted intracranially in rats were treated by TTFields using an outer electrode [8]. The optimal frequency and intensity were reported as 200 kHz and 2 V/cm, respectively, based on cytology. The tumors were measured by MRI after 6 days; the largest diameter tumor in the treatment group was only about half of that in the control group. The effect of TTFields in one direction was negligible, whereas the two-direction and three-direction TTFields reduced the tumor size by 42.6% and 53.4%, respectively, in the treatment group compared with that in the control group. These results were consistent with those observed in vitro studies.

TTFields in animals showed no significant adverse effects. TTFields was applied to the heads and chests of rabbits to test safety. Weight, temperature, electrocardiogram, complete blood count, electrolyte and coagulation function were evaluated over time. All animals were sacrificed after one month. No changes in heart rate and heart rhythm were observed, and no treatment-related toxicity was detected. Tumors were significantly reduced under a specific electric field frequency.

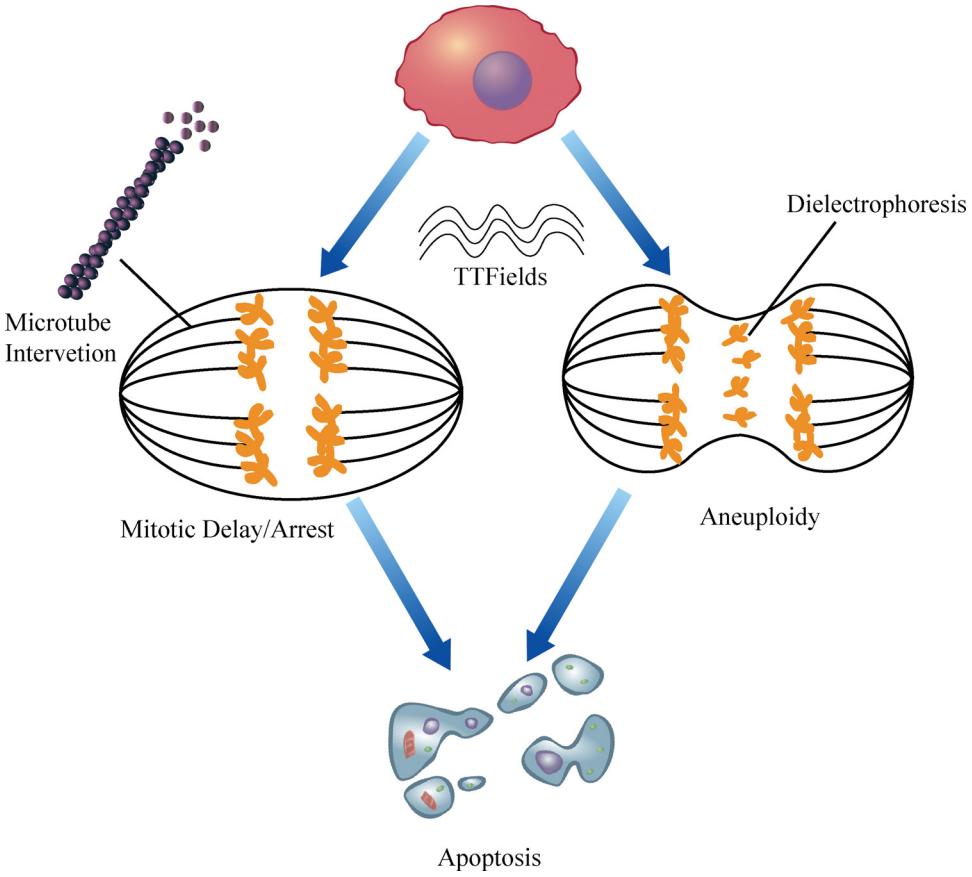

Fig. 3. Schematic representation of two mechanisms of targeting of tumor cells by TTFields. During metaphase in mitosis, the directional forces of TTFields disturb microtubule polymerization and subsequent mitotic delay or arrest, leading to cell death. In addition, the nonuniform field forces all polar molecules to move toward the cleavage ditch, which is termed dielectrophoresis, leading to structural and functional disorder in the cell, which then promotes apoptosis.

4. Clinical studies on TTFields

4.1. Clinical characteristics of TTFields

TTFields is a new, noninvasive tumor treatment option with the following clinical characteristics. (1) An insulating electrode is placed on the skin of the tumor growth site for almost all cancers. The energy between the transducer arrays does not decay; thus, deep malignant tumors can also be treated, such as brain, lung, ovarian, and pancreatic cancers. (2) When combined with conventional radiotherapy and chemotherapy, TTFields can improve the efficacy and sensitivity of the treatment without increasing systemic toxicity [16] and thereby improve the quality of life [17]. (3) TTFields is induced by a transducer array placed on the skin near the tumor. Because the electric field does not have a half-life, sustainable treatment is possible [18]. (4) TTFields is safe and noninvasive with few adverse effects except mild dermatitis. This side effect can be addressed by simple strategies, including shaving, scalp cleaning, frequent transducer array replacement and local corticosteroids [19]. (5) TTFields can also be employed as a wearable device, making such treatment more convenient [20] and thereby improving patient compliance and quality of life. With sufficient social support and independence, patient compliance has been reported to be more than 75% [21]. At present, patient compliance is typically improved through professional guidance and management. However, the optimal frequency for patient management varies by tumor type. These issues should be addressed prior to worldwide use.

4.2. Clinical trials of TTFields in HGG

TTFields has shown great potential in treating HGG. Ten patients with recurrent glioblastoma were first treated with TTFields without being given adjuvant chemotherapy. Compared with the control, the median time of disease progression (median 1TRP) was prolonged (26.1 weeks), the 6-month PFS rate of patients was also improved (50%), and the median OS of patients was more than 62 weeks [10].

Another multicenter randomized open phase III clinical trial (NCT00916409) confirmed that temozolomide (TMZ) combined with TTFields significantly prolonged PFS and OS compared with temozolomide alone. The study included 695 patients with glioblastoma from 83 medical centers in the United States, Canada, Europe, and Israel. The randomized median PFS of TTFields TMZ group and TMZ group alone were 6.7 and 4 months, respectively (HR = 0.66 3. 95% CI 0.52-0.76, $P < 0.001$), with OS 20.9 and 16.0 months (HR = 0.63, 95% CI 0.53-0.76, $P < 0.001$) [22]. The results showed that TTFields could increase the sensitivity of TMZ, and there was no superimposed toxicity. Similarly, in an ef-14 phase III trial subgroup analysis, compared with TMZ alone, TTFields combined with TMZ group in GBM patients was significantly related to improved OS and PFS and the median OS and 5-year survival rate of patients [23]. Moreover, TTFields was safer with no adverse events (AES) related to level 3 or 4 treatment (Fig. 5) [10].

4.3. TTFields combined therapy strategy

TTFields has made remarkable achievements in the field of cancer treatment. TTFields can be combined with chemotherapy, radiotherapy, mitotic checkpoint inhibitors, molecular targeted drugs, PD-1/PD-L1 inhibitors, Ca^{2+} channel antagonists, autophagy inhibitors and other treatment methods to further enhance their therapeutic effects.

Treatment strategies that pair with TTFields include (1) chemotherapy [24]; (2) radiotherapy (TTFields enhances the sensitivity of radiotherapy by suppressing the BRCA1 signaling pathway and reducing DNA self-repair ability) [25]; (3) mitotic checkpoint inhibitors to enhance the anti-HGG effect [26]; (4) targeted therapy, e.g., sorafenib to block G2/M phases, increase the proportion of G0/G1 cells, and significantly inhibit invasion, metastasis, and angiogenesis [27]; (5) immunotherapy, e.g., PD-1/PD-L1 inhibitors for promising lung cancer

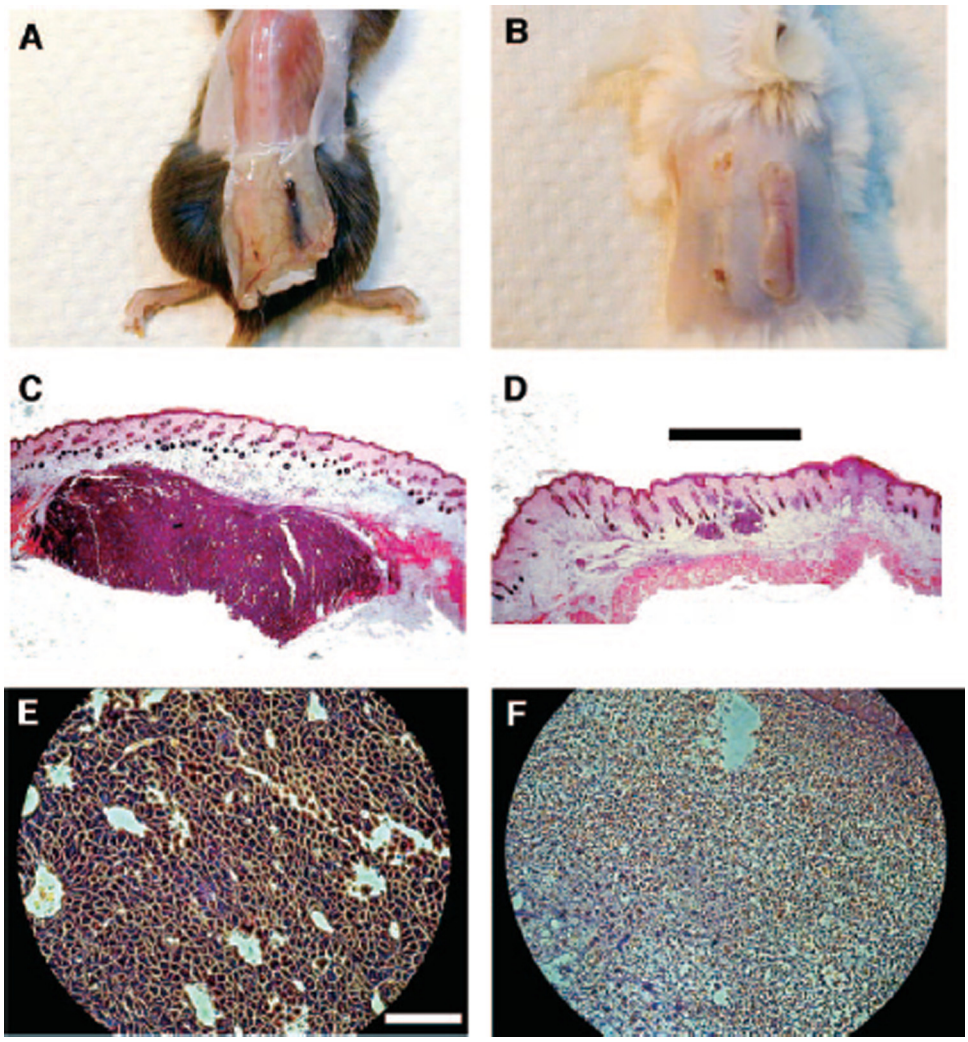

Fig. 4. In vivo effects of TTFields on intradermal tumors in mice. Malignant melanoma (**A**) and adenocarcinoma (**B**) tumor cells were injected in two parallel locations intradermally on the back of each mouse. Only the tumor on the left side of the mouse was treated. After 4 days of TTFields treatment (at 100 kHz), no tumor can be discerned on the treated side, whereas on the untreated side, a large tumor has grown. **C–F**, histological sections of TTFields-treated intradermal melanoma versus a control (untreated) melanoma on the same mouse. **C**. After H&E staining, a large (5 mm-diameter) nodule of melanoma cells can be seen in the dermis of the control tumor (X40). Note that due to the large size of the tumor, the deeper portion was lost during preparation. **D**. Treated tumor; only two small (< 0.4 mm-diameter) nodules are present (scale bar = 0.5 mm). The nontumor structures of the dermis are morphologically intact. **E**. Control tumor; malignant melanoma cells appear intact and viable (X200) (Scale bar = 100 m). **F**. Only necrotic tissue and cellular debris are seen in the treated tumor. Adapted from Kirson ED., et al. (8).

treatment; (6) Ca^{2+} channel antagonists (e.g., the Cav1.2 channel is the target of TTFields in HGG cell plasma membrane) [28]; (7) autophagy inhibitors, such as chloroquine, which can significantly reduce dose-dependent anti-cell growth compared with chloroquine alone [29]; and (8) monoclonal antibodies, such as bevacizumab, which is well tolerated and has shown clinical efficacy [30,31].

4.4. Adverse effects and strategies

There are four main types of adverse skin effects resulting from TTFields: dermatitis (allergy or irritation), erosion, infection, and ulcer. Kirson et al. reported that 9 patients (90%) had mild to moderate adverse skin effects; the patients were cured after local administration of glucocorticoids [10]. By comparison, the incidences of adverse effects in the blood and digestive systems of the TTFields group were only 3% and 4%, respectively, and the incidence of infection was 4% [32]. Compared with TMZ monotherapy, the addition of TTFields did not lead to any significant differences in the overall incidence, distribution, and severity of adverse events except localized skin toxicity. The incidence of nervous system disorders was similar between the two groups (22% in the TTFields group vs. 25% in the control group) [33]. The researchers also evaluated the quality of life of patients; compared with the TMZ group, TTFields group showed no significant differences. However, the TTFields group was significantly better than the TMZ group in cognitive, ritual, role and emotional functions. Furthermore, the frequency and intensity of adverse effects in TTFields group were significantly reduced [34].

Antibiotics are recommended when the epithelial barrier is damaged (eroded) or infected. Because topical antibiotic ointments contain lipid components, ointment residue left on skin must be cleaned. Otherwise, the residue will negatively prevent complete contact between the electrode and the skin, thereby weakening the TTFields treatment. The use of antibiotics depends on the type of bacteria, such as mupirocin or polymyxin B ointment. TTFields treatment for 2-7 days usually cures adverse skin effects, which is consistent with the renewal period of epidermal cells. For severe adverse skin effects, TTFields treatment may be temporarily suspended. Patients with a history of adverse skin effects are prone to relapse; thus, patient education and prevention are essential.

4.5. TTFields treatment compliance

The efficacy of TTFields is primarily limited by patient compliance. Unlike chemotherapy, TTFields treatment is physical and has no half-life. Therefore, any anti-cancer effects disappear once a TTFields device is turned off. Thus, TTFields treatments require the use of wearable devices [14]. TTFields treatments require at least 4 weeks before any substantial tumor growth reversal is observed [13]. Electrodes need to be installed on a shaved scalp. The correct installation of electrodes requires professional training. Researchers hope to increase battery life and reduce device size and weight to achieve better patient compliance. Some patients cease TTFields treatment because of contact dermatitis. However, the correct preparation of the skin and local application of antibiotics and glucocorticoid ointments can reduce dermatitis and

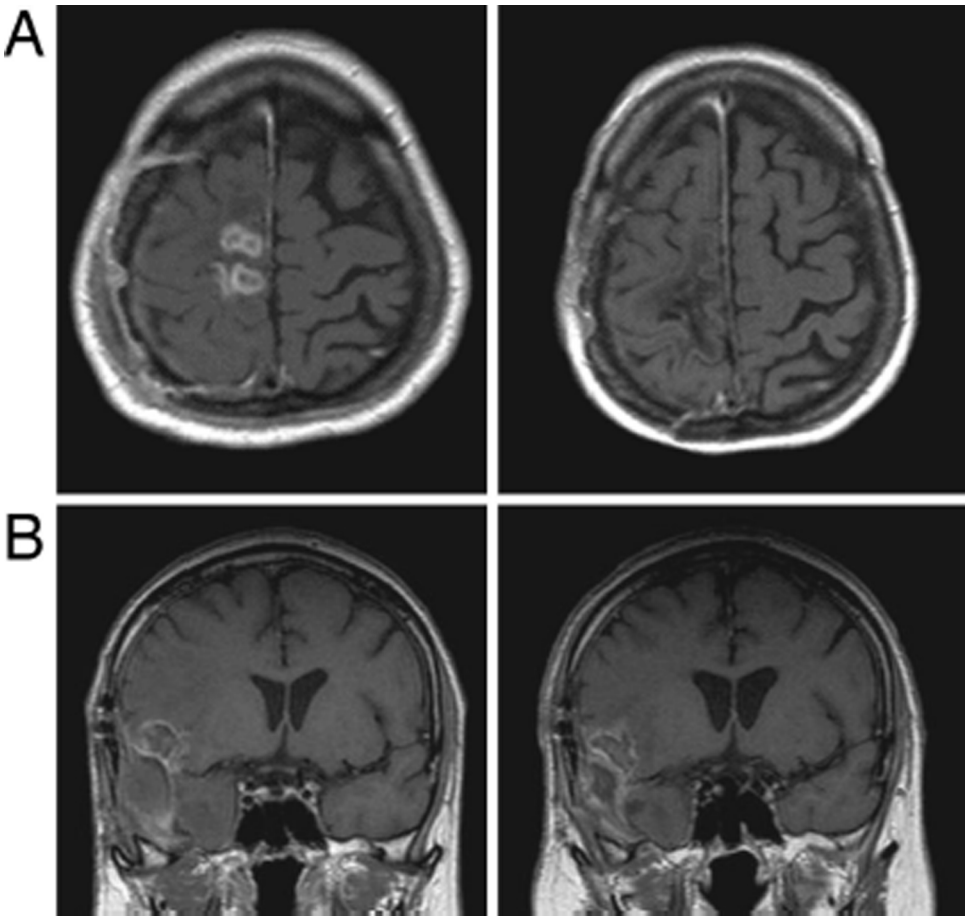

Fig. 5. Examples of T1-weighted, post-contrast MRI scans of recurrent GBM patients before (left) and after (right) TTFields treatment. **A**, Complete response after 8 months of treatment. **B**, Stable disease (10% reduction in the contrast-enhanced area) after 9 months of treatment. Adapted from Kirson, E.D., et al. (26). Copyright (2007) National Academy of Sciences.

improve compliance [35].

4.6. Resistance of TTFields

In theory, the resistance rate of TTFields is lower than that of standard chemotherapy. Additionally, mitosis is a crucial physiological activity for tumor cell proliferation. The multidrug resistance of HGG is a common phenomenon after long-term chemotherapy. Furthermore, the blood-brain barrier, which limits the effects of chemotherapy drugs, poses no issue for TTFields therapy. In a phase 3 randomized clinical study, patients treated with TTFields showed significant radiologic responses, with only 14% of patients indicating tumor resistance. The possible mechanism of HGG resistance to TTFields is likely correlated with cell volume [10]. Turner et al. reported on the treatment of glioblastoma with TTFields and chemotherapy; tumor volume increased instead of decreased [36]. As confirmed in ovarian cancer research, tumor resistance can be reversed by reducing the frequency of TTFields [37]; this suggests the importance of imaging review and biopsy to decide whether to adjust the frequency of TTFields treatment. TTFields therapy has been shown to induce mutations in tumor cells; thus, TTFields resistance may be the result of mutations in chromosome repair.

4.7. Disputes over TTFields treatment

Although the systemic adverse effects of TTFields treatment are few, specific populations are not suitable for the therapy. No studies have indicated that TTFields does not interfere with implanted electronic devices (e.g., pacemakers, defibrillators and deep brain stimulators). TTFields has been reported to be well-tolerated in a patient who received general valve ventriculoperitoneal drainage; however, no systematic studies have been conducted to assess the effect of TTFields on the ventriculoperitoneal shunt valve. TTFields treatment is prohibited in any patient allergic to hydrogels coating the transducer. Moreover, the practical application of TTFields is limited due to its high expense. A recent study evaluated the cost-effectiveness of TTFields in newly diagnosed patients with GBM by calculating the costs per life-years gained (LYG). The results showed that adding TTFields to standard therapy increases life expectancy at a cost of €510,273/LYG [38].

5. Conclusions

Currently, the National Comprehensive Cancer Network (NCCN) has added TTFields to the list of recommended therapies for the treatment of recurrent or drug-resistant tumors. TTFields interferes with mitotic spindle formation and disrupts cell structure by inducing DEP, leading to the abnormal proliferation and apoptosis of tumor cells. Several studies have shown the anti-tumor activity of TTFields in different tumor cells. These studies have also shown that different parameters, including frequency and intensity, result in different efficacies in specific cells. Additional studies are necessary to determine the optimal TTFields parameters for each glioma subtype (Fig. 6). At present, TTFields technology has only been applied to glioblastoma. It has been reported that TTFields treatment could alter the morphology and inhibit the proliferation of patient-derived anaplastic meningioma cell lines [39], indicating the potential for TTFields to be utilized for other central nervous system tumors.

Future studies are warranted to monitor the effects of TTFields on normal glial cells and neurons. TTFields treatment has been shown to affect the activity of neural stem cells, interfere with their differentiation and proliferation, promote neuron oscillation, stimulate the growth of neural processes and accelerate healing [40]. At the same time, the

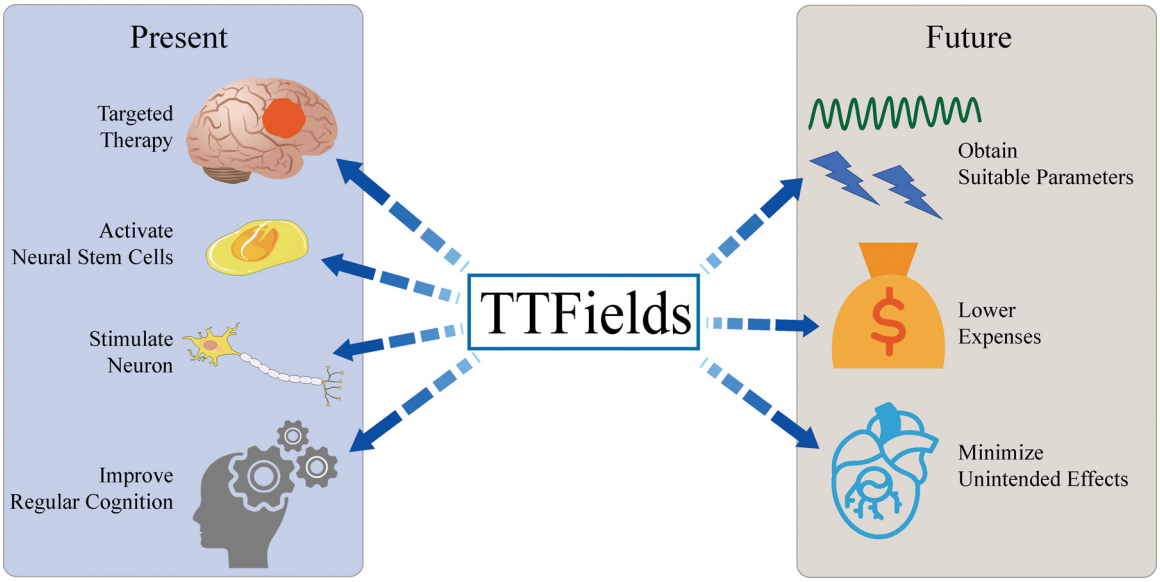

Fig. 6. Perspectives of TTFields during clinical applications. Currently, several studies have demonstrated that the application of TTFields could target malignancies other than glioma, activate neural stem cells, stimulate the biological activities of neurons, and improve cognitive functions. Further studies are warranted to explore suitable parameters for targeting different cancers, to reduce TTF device expenses to achieve a high cost-effectiveness and to minimize unpredictable effects on implanted electronic devices in patients.

current of TTFields has been found to alter neuronal activity by changing the discharge rate, excitation characteristics and peak times [28]. Therefore, the effects of TTFields therapy on memory, learning, and other cognitive functions should be carefully evaluated. However, due to the poor prognosis of glioblastoma patients and the good clinical prospects of TTFields, such problems should not impede the clinical application of TTFields. The combination of TTFields with standard therapy would provide the best treatment for patients with new diagnoses and recurrent gliomas by improving the prognosis and reducing the incidence of adverse effects. However, TTFields is prohibitively expensive and has unpredictable effects on implanted devices. Thus, the application of TTFields should be carefully considered. Overall, TTFields can be used to treat tumors with promising efficacy, few adverse effects, and high cost-effectiveness.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

Acknowledgments

Not applicable.

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