# Tumor-treating fields in combination with sorafenib restrain the proliferation of liver cancer *in vitro*

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**Abstract.** Liver cancer is a common malignancy worldwide, with a poor prognosis and a high recurrence rate despite the available treatment methodologies. Tumor-treating fields (TTFields) have shown good preclinical and clinical results for improving the prognosis of patients with glioblastoma and malignant pleural mesothelioma. However, there is minimal evidence for the effect of TTFields on other cancer types. Thus, the present study aimed to investigate the therapeutic efficacy of TTFields in an *in vitro* model, and to further elucidate the underlying mechanisms. In the present study, two hepatocellular carcinoma (HCC) cell lines (Hep3B and HepG2) were treated with TTFields (intensity, 1.0 V/cm; frequency, 150 kHz) in order to determine the potential anti-tumor effects of this approach. TTFields significantly inhibited the proliferation and viability of HCC cell lines, as measured using Trypan blue and MTT assays, as well as colony formation in three-dimensional cultures. The TTFields also significantly inhibited the migration and invasion of HCC cells in Transwell chamber and wound-healing assays. Moreover, TTFields enhanced the production of reactive oxygen species in the cells and increased the proportion of apoptotic cells, as evidenced by increased caspase-3 activity, as well as PARP cleavage in western blotting experiments. All of these effects were increased following the application of TTFields in combination with the multi-kinase inhibitor sorafenib, which demonstrated a synergistic effect. Thus, to the best of our knowledge, these results demonstrate for the first time the potential of TTFields in improving the sensitivity of HCC cells to sorafenib, which may lay the foundation for future clinical trials for this combination treatment strategy.

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## Introduction

Hepatocellular carcinoma (HCC) is one of the most frequently occurring malignant tumors, which accounts for >80% of liver cancer cases (1,2). HCC can be generally subclassified as primary and secondary malignant HCC, and the former type has a poor prognosis, with a particularly high mortality rate in China (1,2). The current treatment options for HCC include surgical resection, chemotherapy, radiation therapy and gene therapy (3). However, despite the rapid progress in technology yielding new candidate diagnostic and therapeutic approaches, the curative rate for HCC remains poor with an estimated 5-year survival rate of only 12% (3). To overcome this challenge, new treatment strategies are needed, thus necessitating improved understanding of HCC progression.

Tumor-treating fields (TTFields) are low-intensity, intermediate-frequency alternating electric fields, which can act on rapidly dividing glioma and other cancer cells, especially during the metaphase, anaphase and telophase stages of mitotic cell division (4). With the application of an alternating electric field, charged molecules within the cancerous cells start to oscillate along with the rotation of the dipoles (5). Under the alternating electric field, molecules with a high electrical dipole moment such as tubulin dimers and septins are forced to align with the direction of the alternating electric fields. Since these molecules are the polymers generated in a mitotic cell during metaphase (6,7), the TTFields disrupt the microtubule spindle formation and the localization of septin fibers, leading to mitotic catastrophe and ultimately mitotic cell death (8). Nevertheless, a high number of cells can still pass through this stage and enter the subsequent anaphase and telophase stages, in which the mitotic cell assumes an hourglass shape through the formation of a cleavage furrow in the center to facilitate the formation of daughter cells. An alternative electric field disrupts this new shape, causing the polarized components to move toward the furrow (dielectrophoresis), ultimately

obstructing the entire process of mitosis (8-10). Therefore, the application of TTFields can lead to death or to the formation of abnormal dividing cells with an unusual number of chromosomes (9,10).

Preclinical studies have demonstrated increased sensitivity to chemotherapy in combination with TTFields, both in human glioblastoma (GBM) cell lines and in animal tumor models (9-12). In addition, a synergistic effect was observed between TTFields and radiotherapy, which may benefit patients with GBM (13,14).

Clinical trials have also shown that patients with recurrent GBM can benefit from TTFields alone, as this treatment prolonged their overall survival without complications (15). Furthermore, the common TTFields side effects were not observed, except for medical device site reaction headache and muscle twitching, and the main side effect for TTFields is mild to moderate skin irritation underneath the transducer arrays. Further, common TTFields side effects did not show except included medical device site reaction, headache and muscle twitching (16). However, TTFields technology has evolved in recent years to achieve improved results, leading to its approval by the US Food and Drug Administration. Currently, TTFields are regarded as an alternative to the standard treatment for patients with recurrent GBM designated as National Comprehensive Cancer Network category 1 and has also received a Communauté Européenne certification mark in Europe (17).

The preclinical and clinical data of the effects of TTFields are currently mainly available for GBM and are being studied for other cancer types. Therefore, in the present study, the effects of TTFields were evaluated in liver cancer cells for their ability to inhibit proliferation.

## Materials and methods

*Experimental setup of the electric fields*. TTFields were generated using a pair of insulated wires connected to a functional generator and a high-voltage amplifier, which generated sine-wave signals ranging from 0 to 800 V (18). The setup resulted in an applied electric field intensity and frequency of 0.9 V/cm and 150 kHz, respectively. A field intensity of 1.0 V/cm was used due of its use in clinical settings. For irradiation, cells were plated in 100-mm dishes and incubated at 37˚C in a humidified atmosphere containing 5% $CO_2$ until they reached 70-80% confluence.

*Cell culture*. The human hepatocarcinoma Hep3B cells were obtained from the Korean Cell Line Bank (KCLB; cat. no. 88064) and were cultured in DMEM supplemented with 10% heat-inactivated FBS (both Gibco; Thermo Fisher Scientific, Inc.), 0.1 mM non-essential amino acids, glutamine, HEPES and antibiotics at 37˚C in a 5% $CO_2$ humidified incubator. The human HepG2 hepatoblastoma cell line was obtained from the KCLB (cat. no. 88065) and grown in RPMI-1640 medium supplemented with 10% FBS, glutamine, HEPES and antibiotics at 37˚C in a 5% $CO_2$ humidified incubator.

*Cell viability assay*. Liver cancer cells were treated with TTFields (1.0 V/cm; 150 kHz), 5 $\mu$M sorafenib (Selleck Chemicals) or a combination of both for 24 h, and cell viability was determined using a Trypan blue exclusion assay. An equal volume of Trypan blue reagent was added to the cell suspension, and the percentage of viable cells was evaluated under a light microscope (Olympus CK40; Olympus Corporation). The assays were performed in triplicate.

*Colony-forming assay*. Hep3B and HepG2 cells (500-1,000) were seeded into 6-well plates in triplicate and treated with TTFields (1.0 V/cm; 150 kHz), sorafenib (5 $\mu$M) or both concurrently for 72 h. After 10-14 days, colonies were fixed with 100% methanol for 30 min and stained with 0.4% crystal violet (Sigma-Aldrich; Merck KGaA) according to the manufacturer's instructions (11). The plating efficiency (PE) represents the percentage of seeded cells that grew into colonies under the specific culture conditions of a given cell line. The survival fraction was calculated as follows: Survival fraction=colonies counted/(cells seeded x PE/100). Colonies are counted using imaging analysis software.

*Cell death detection*. Cell death was analyzed in the Hep3B and HepG2 cell lines 72 h after concurrent treatment with TTFields (1.0 V/cm; 150 kHz) and 5 $\mu$M sorafenib using a Cell Death Detection ELISA kit (Roche Diagnostics GmbH). Cells were treated, harvested and stained with cell death detection reagent according to the manufacturer's protocol (19). The absorbance was then measured using a Multiskan EX plate reader (Thermo Fisher Scientific, Inc.) at 450 nm.

*Caspase3 activity assay*. Caspase-3 activity was analyzed in the Hep3B and HepG2 cell lines 72 h after concurrent treatment with TTFields (1.0 V/cm; 150 kHz) and 5 $\mu$M sorafenib using a Caspase-Glo 3/7 assay detection kit (cat. no. G8091; Promega Corporation). The assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (*p*NA) after cleavage from the labeled substrates of DEVD-*p*NA (for caspase-3). The *p*NA light emission was quantified using a Multiskan EX plate reader (Thermo Fisher Scientific, Inc.) at 405 nm. Comparison of the *p*NA absorbance of apoptotic and control samples allows determination of the fold increase in caspase activity.

*Reactive oxygen species (ROS) detection*. ROS generation was analyzed in the Hep3B and HepG2 cell lines 6 h after treatment with TTFields (1.0 V/cm; 150 kHz) and 5 $\mu$M sorafenib. The cells were cultured, harvested at the indicated times, according to the manufacturer's protocol (20) and ROS levels were then measured using a Multiskan EX plate reader (Thermo Fisher Scientific, Inc.) at 450 nm (20). ROS was monitored using the fluorescent ROS indicator, C2',7'-dichlorodihydrofluorescein diacetate (5 $\mu$M; Molecular Probes). N-acetyl cysteine (NAC) was obtained from Sigma-Aldrich (Merck KGaA), and Hep3B and HepG2 cell lines were subsequently treated with NAC (10 mM) and the indicated concentration of sorafenib or TTFields. The production of ROS was estimated using FACS analysis with DCFDA staining. The data were acquired using a FACSort™ flow cytometer with CellQuest™ software (version 7.5.3; both from BD Biosciences).

*Three-dimensional (3D) culture system*. Hep3B and HepG2 cells were seeded in 96-well plates at a density of

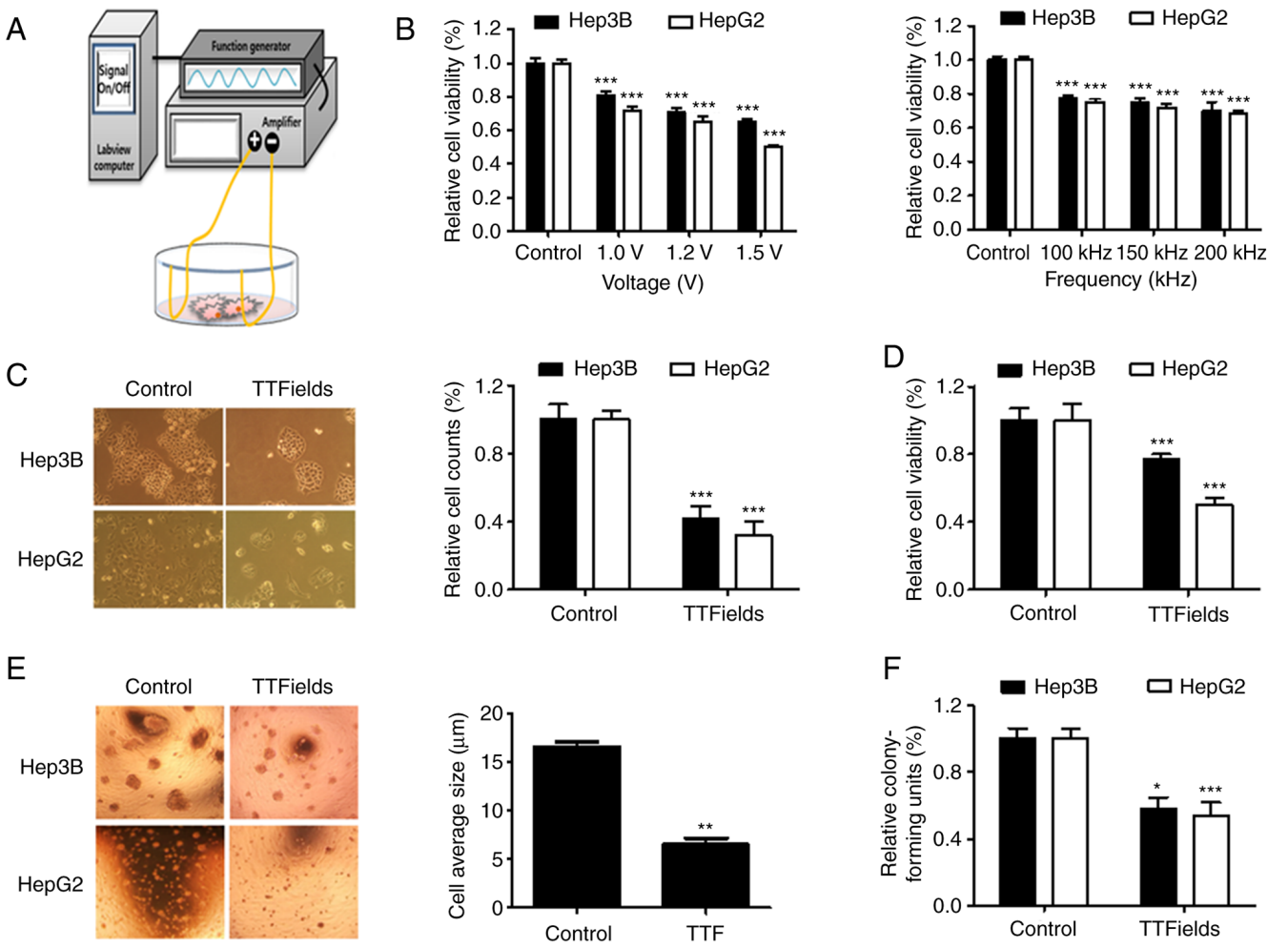


Figure 1. Effect of TTFields on the viability of liver cancer cells. (A) Experimental scheme of treatment with TTFields in liver cancer cell lines. (B) The analysis of liver cancer cell viability analysis according to the frequency and the intensity. (C) The viability of cells treated with TTFields was significantly lower than that of control cells. The proliferation rate was detected by cell counting. Representative microscopic images (magnification, x10) and relative cell counts. (D) MTT assay after application of TTFields for 48 h. (E) 3D colony culture after application of TTFields for 48 h. (F) Sensitivity of liver cancer cells treated with TTFields (1.0 V/cm; 150 kHz) was measured using a colony formation assay. Representative microscopic images (magnification, x40), $^{*}$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001 vs. respective controls. TTFields/TTF, tumor-treating fields.

$1x10^4$ cells/well. In the 3D culture model, the 96-well plates were pre-coated with 40 $\mu$l Matrigel® basement membrane, then incubated at 37˚C for 30 min. The cells were plated on the gel in an appropriate medium (10% heat-inactivated FBS, 0.1 mM non-essential amino acids, glutamine, HEPES and 1% (v/v) penicillin-streptomycin (Gibco®, Life Technologies), and the wells were examined using a light microscope (Olympus CK40; Olympus Corporation) after a duration of 10 days (21).

*Transwell chamber assay*. The migration and invasion of liver cancer cells were measured using Transwell chambers according to the manufacturer's protocol and as described previously (21). Briefly, the cells were seeded onto the membrane of the upper chamber at a concentration of $4x10^5$ cells/ml in 150 $\mu$l serum-free medium and were either left untreated or treated with TTFields for 24 h. The medium in the lower chamber contained 10% (v/v) FBS as a source of chemoattractants. For the invasion assay, cells that passed through the Matrigel®-coated membrane (coating time, 30 min at 37˚C) were stained with Cell Stain Solution containing Crystal violet (MilliporeSigma) for 30 min, and for the migration assay, cells that passed through the gelatin-coated membrane were stained as previously described and examined after 24-h incubation. The wells were evaluated under a light microscope (Olympus CK40; Olympus Corporation).

*Western blot analysis*. Total proteins from liver cancer cells were extracted in RIPA buffer [50 mM Tris-Cl (pH 7.4), 1% NP-40, 150 mM NaCl and 1 mM EDTA] supplemented with protease inhibitors (1 mM PMSF, 1 $\mu$g/ml aprotinin, 1 $\mu$g/ml leupeptin and 1 mM $Na_3VO_4$) and quantified using the Bradford method. Protein samples (30 $\mu$g) were separated using SDS-PAGE on an 11% gel and subsequently transferred to a nitrocellulose membrane. After blocking non-specific antibody binding sites using 5% skim milk diluted in 1X TBS with 0.1% Tween-20 for 1 h at room temperature, the membrane was incubated overnight at 4˚C with primary antibodies against poly (ADP-ribose) polymerase (1:1,000; PARP; cat. no. 9542), cleaved PARP (1:1,000; cat. no. 9541), caspase-3 (1:1,000; cat. no. 9662), cleaved caspase-3 (1:1,000; cat. no. 9664) and MMP9 (1:1,000; cat. no. 3852), all purchased from Cell Signaling Technology, Inc. Anti-β-actin (1:200; cat. no. sc-47778)

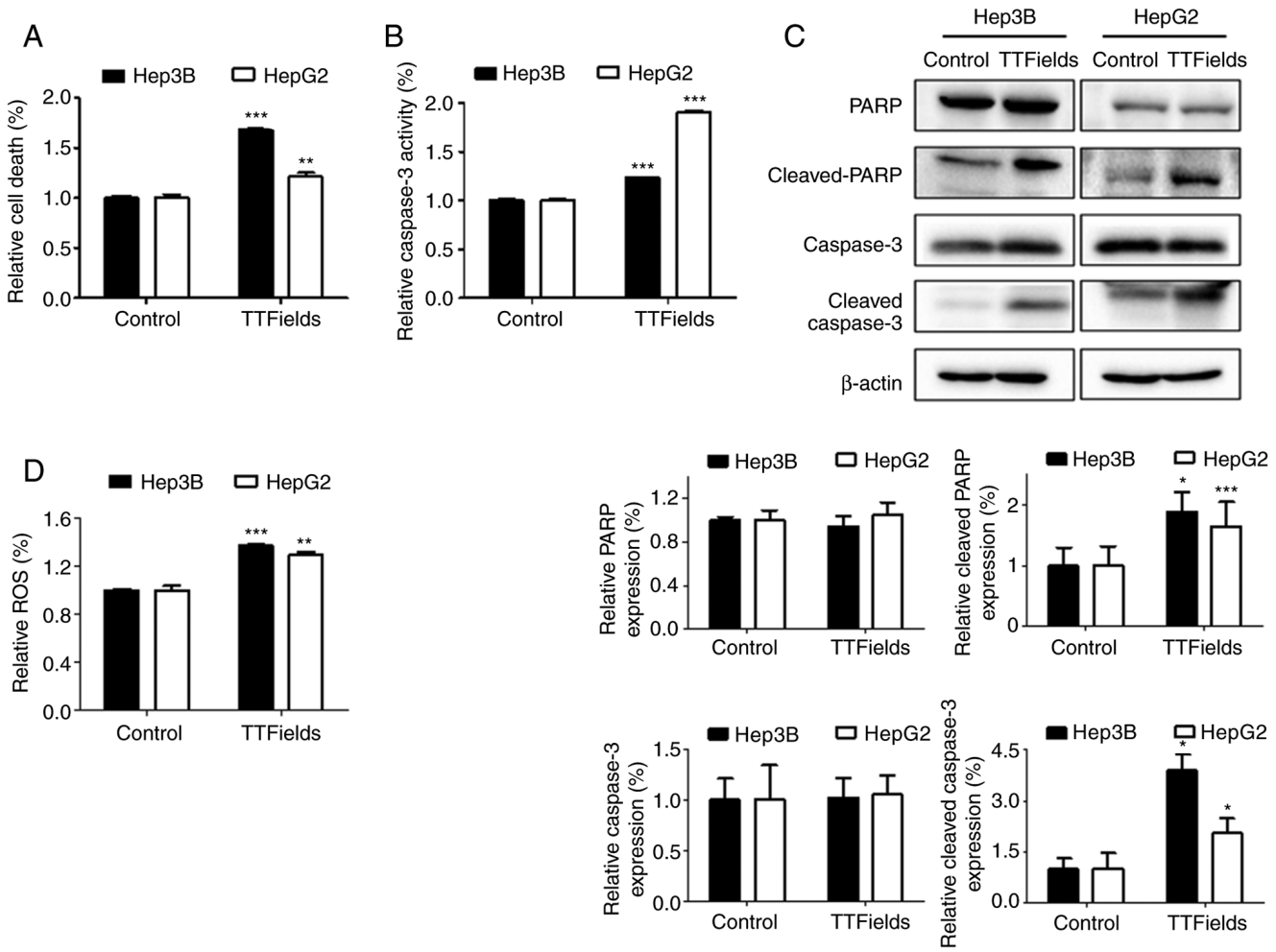


Figure 2. Effect of TTFields on the apoptosis of liver cancer cells. (A) Analysis of cell death in liver cancer cell lines 72 h after treatment with TTFields (1.0 V/cm; 150 kHz) using a cell death detection kit. $^{**}$P<0.01, $^{***}$P<0.001 vs. respective controls. (B) Analysis of caspase activity in liver cancer cell lines 72 h after treatment with TTFields (1.0 V/cm; 150 kHz) using ELISA. $^{***}$P<0.001 vs. respective controls. (C) Cell lysates (30 $\mu$g) were immunoblotted with the indicated antibodies. $^{*}$P<0.05, $^{***}$P<0.001 vs. respective controls. (D) Analysis of ROS generation in liver cancer cell lines 72 h after treatment with TTFields (1.0 V/cm; 150 kHz) using an ROS detection kit. $^{**}$P<0.01, $^{***}$P<0.001 vs. respective controls. TTFields/TTF, tumor-treating fields; ROS, reactive oxygen species; PARP, poly (ADP-ribose) polymerase 1.

was purchased from Santa Cruz Biotechnology, Inc. After incubation with the following peroxidase-conjugated secondary antibodies: Mouse anti-rabbit IgG-HRP (1:5,000; cat. no. sc-2357; Santa Cruz Biotechnology, Inc.) and goat anti-mouse IgG-HRP (1:5,000; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) at 37˚C for 1 h, the protein bands were visualized using enhanced chemiluminescence reagent (GE Healthcare Life Sciences) and detected using the Amersham Imager 680 (version, 2.0; GE Healthcare Life Sciences).

*Statistical analysis*. Statistical significance was determined using two-way ANOVA followed by tukey's post hoc test. Values represent the mean of three experimental repeats ± standard deviation. Data analysis was performed using the GraphPad Prism 6 software (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

## Results

*Effect of TTFields on liver cancer cell proliferation*. The TTFields setup was constructed using insulated wires connected to a generator and an amplifier to generate a sine-wave signal ranging from 0 to 800 V (Fig. 1A). To determine the optimal TTFields voltage and frequency, Hep3B and HepG2 cells were subjected to various conditions (Voltage, 0, 1.0, 1.2 and 1.5 V/cm; frequency, 0, 100, 150 and 200 kHz) for 48 h (Fig. 1B). The two liver cancer cell lines exhibited a voltage-dependent reduction in cell viability (~20% at 1.0 V/cm; 150 kHz). To evaluate TTFields-induced cytotoxicity and a cell viability assay was performed using Hep3B and HepG2 cells. Application of TTFields for 48 h resulted in a significant reduction in the proliferation of Hep3B and HepG2 cells, as determined by the Trypan blue (Fig. 1C) and MTT assays (Fig. 1D). Furthermore, the colonies in untreated 3D cultures (size, 17 $\mu$m) were significantly larger than those formed by TTFields-treated cells (size, 6 $\mu$m; Fig. 1E). The survival fraction showed a clonogenic efficiency with a reduction of 42% in Hep3B cells and of 46% in HepG2 cells following treatment (Fig. 1F). Collectively, these findings suggest that TTFields can inhibit the proliferation of liver cancer cells.

*TTFields enhances the apoptosis of liver cancer cells*. To investigate whether the apoptosis of liver cancer cells was

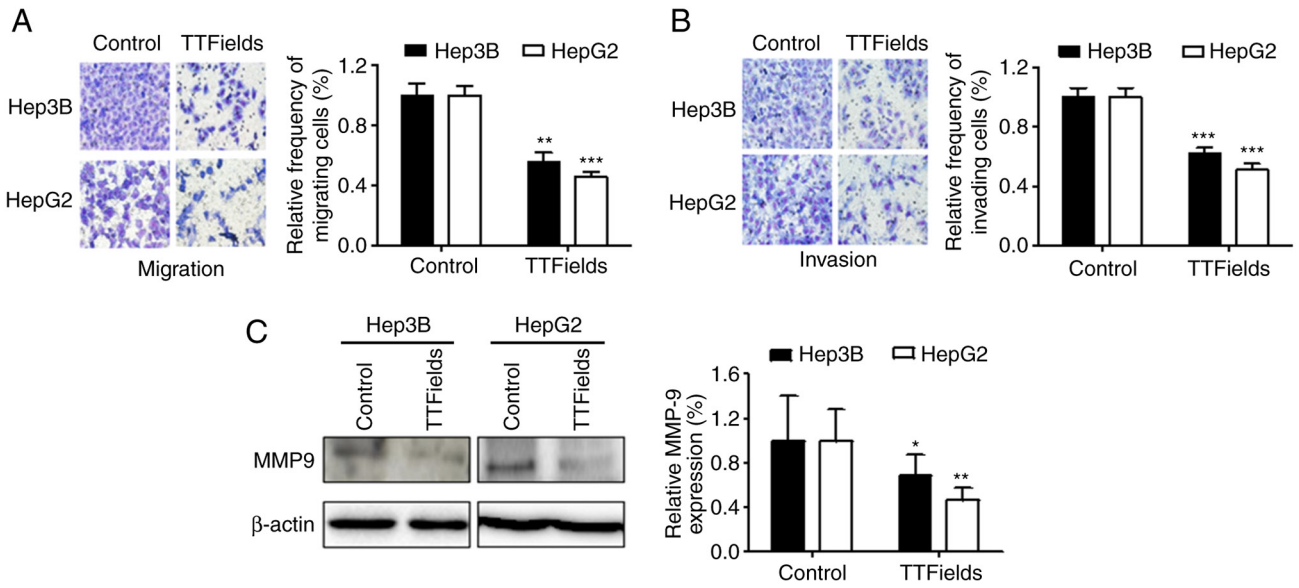


Figure 3. TTFields inhibit the migration and invasion of liver cancer cells. (A and B) Tumor cell migration and invasion after 24 h TTFields (1.0 V/cm; 150 kHz) treatment examined using Transwell chamber assays. The number of invading tumor cells that penetrated through the Matrigel/gelatin was counted in five high-intensity fields. Representative microscopic images (magnification, x40), $^{**}$P<0.01, $^{***}$P<0.001 vs. respective controls. (C) Cell lysates (30 $\mu$g) were immunoblotted with the indicated antibodies. TTFields/TTF, tumor-treating fields. $^{*}$P<0.05, $^{**}$P<0.01 vs. respective controls.

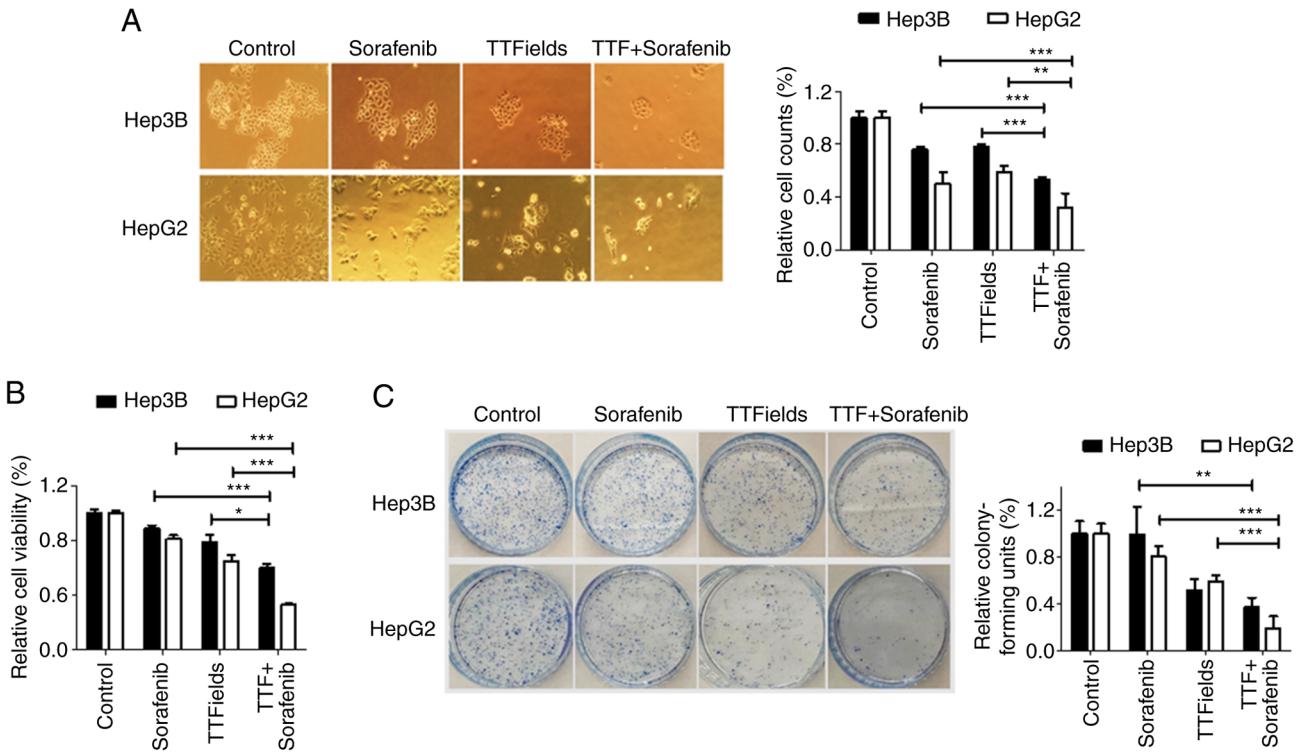


Figure 4. TTFields sensitizes liver cancer cells to sorafenib. (A and B) Liver cancer cells were treated with TTFields (1.0 V/cm; 150 kHz), sorafenib (5 $\mu$M) or both concurrently for 24 h, and cell viability was determined using a trypan blue exclusion assay. (A) Representative microscopic images (magnification, x10) (left) and cell counts (right). $^{**}$P<0.01, $^{***}$P<0.001 vs. respective controls. (B) MTT assay. $^{*}$P<0.05, $^{***}$P<0.001 vs. respective controls. (C) Colony forming assay. $^{**}$P<0.01, $^{***}$P<0.001 vs. respective controls. TTFields/TTF, tumor-treating fields.

induced by the TTFields, early apoptosis was assessed using a cell death detection kit. Exposure to TTFields for 72 h significantly increased the proportion of apoptotic cells in both liver cancer cell lines (Fig. 2A). It was also hypothesized that enhanced TTFields-induced cytotoxicity resulted from the activation of the chief executioners of cell death, caspase-3 and PARP fragmentation (22). The results demonstrated increased caspase-3 activity and PARP cleavage in response to TTFields compared with the control group (Fig. 2B and C). In addition, the production of ROS significantly increased in

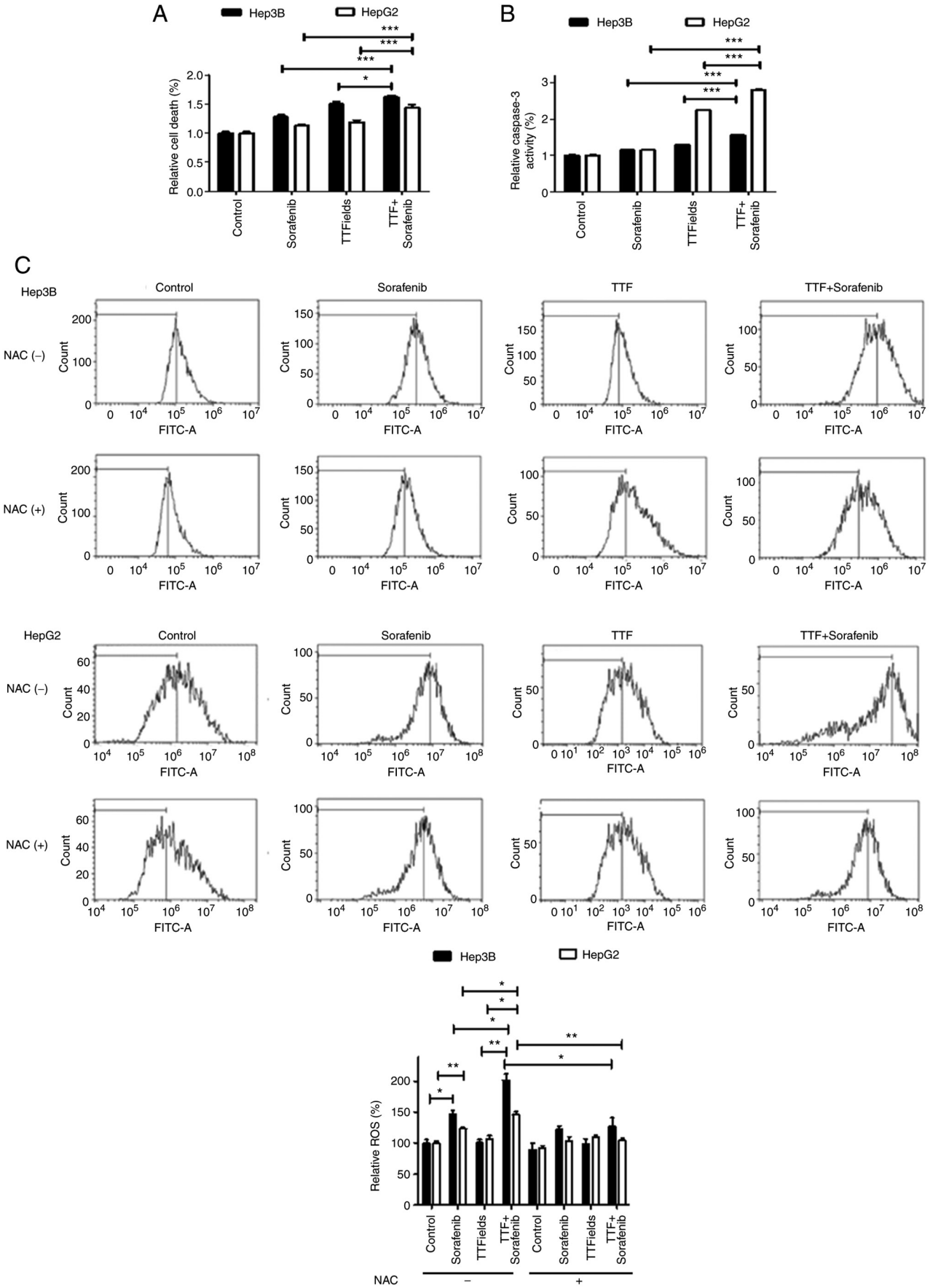


Figure 5. Effect of TTFields combined sorafenib on the apoptosis of liver cancer cells. (A) Analysis of cell death in two liver cancer cell lines 72 h after concurrent treatment with TTFields (1.0 V/cm; 150 kHz) and sorafenib (5 $\mu$M) using a cell death detection kit. $^{*}$P<0.05, $^{***}$P<0.001 vs. respective controls. (B) Analysis of caspase activity in the two liver cancer cell lines 72 h after treatment with TTFields and sorafenib using ELISA. $^{***}$P<0.001 vs. respective controls. (C) Analysis of ROS generation in two liver cancer cell lines 6 h after treatment with TTFields (1.0 V/cm; 150 kHz) using flow cytometry. $^{*}$P<0.05, $^{**}$P<0.01 vs. respective controls. TTFields/TTF, tumor-treating fields; NAC, N-acetylcysteine; ROS, reactive oxygen species.

both cells lines following TTFields application (Fig. 2D). These results indicate that ROS generated by the TTFields treatment increases intracellular caspase signaling leading to apoptosis.

*TTFields suppresses cell migration and invasion*. TTFields treatment has been shown to significantly inhibit tumor cell migration and invasion (21). Therefore, the effect of TTFields on the invasive and migratory capacities of liver cancer cells was examined using Transwell chamber assays. The results demonstrated that TTFields significantly inhibited cell migration compared with the control group (Fig. 3A). Similarly, TTFields treatment inhibited the invasion of both liver cancer cell lines (Fig. 3B). Notably, TTFields also downregulated the expression of MMP9 in liver cancer cells (Fig. 3C).

*Sorafenib sensitizes liver cancer cells to TTFields*. TTFields were applied to Hep3B and HepG2 cells in combination with the multi-kinase inhibitor sorafenib (Fig. 4A). In previous studies, to evaluate the effects of sorafenib on TTFields-induced cytotoxicity, a 5-$\mu$M sorafenib concentration was used, which resulted in 25% growth inhibition after a 48-h exposure in each experiment (23,24). In the present study, after 48 h, the combination of sorafenib and TTFields resulted in a significantly greater antitumor effect on Hep3B and HepG2 cells than either treatment alone, as evidenced by the Trypan blue exclusion assay and the MTT assay (Fig. 4A and B). Moreover, in the colony formation assay, the relative colony forming units were further decreased in cells treated with TTFields and sorafenib than those in cells receiving either of these treatments alone (Fig. 4C). These results indicated that sorafenib sensitized liver cancer cells to TTFields *in vitro*.

*Sorafenib enhances TTFields-induced apoptosis*. To investigate whether sorafenib and TTFields could induce apoptosis, early apoptosis was assessed using cell death kit. In both liver cancer cell lines, 48 h of exposure to sorafenib and TTFields resulted in a significant increase in the proportion of early apoptotic cells (Fig. 5A). Apoptotic cell death significantly increased following combined treatment. To examine the underlying pathway, the activity of caspase 3 was measured, in order to determine whether increased activity of this protein mediates the cytotoxicity of the combined therapy. The results demonstrated a significant increase following combined treatment compared with sorafenib alone (Fig. 5B). ROS production following TTFields application alone or with sorafenib was also evaluated. ROS production significantly increased following combined treatment compared with single treatments (Fig. 5C), which may explain the increased apoptotic rate following combined treatment. These data were further confirmed by incubating the cells with NAC, a ROS scavenger, which abolished the release of ROS following combination treatment in both liver cancer cell lines.

## Discussion

Combination therapy, consisting of maximal safe surgical resection followed by combined chemo-radiotherapy and adjuvant temozolomide, currently represents the standard of care for patients with different forms of cancer. Developing an understanding of the TTFields-oriented approach requires familiarity with two concepts. Firstly, electric fields can be uniform (i.e., an electric field that remains unchanged at all points) or non-uniform (i.e., tends to vary in direction (divergent/convergent) and magnitude). Secondly, it is possible for an electric field to remain in an unchanging field, wherein the constant state of source charge allows a test charge to converge unidirectionally (4,5,6,7). To inhibit the growth of cancerous cells, both their proliferation and apoptosis need to be considered. In the present study, the findings of western blot analysis and ROS assays suggested that, in comparison with monotherapy, TTFields combined with sorafenib demonstrated greater anti-proliferative and apoptosis effects on liver cancer cells. Moreover, Transwell migration and invasion assays demonstrated that TTFields combined with sorafenib inhibited liver cancer cell invasion and metastasis synergistically.

Preclinical studies have demonstrated that the optimal anti-proliferative effect of TTFields on isolated cancer cells is dependent on the frequency of the electric fields specific to the source of the isolated tumor cells (9,11,12,25). In clinical settings, TTFields was applied at 200 kHz for GBM, which was the frequency demonstrating the greatest decrease in glioma cell proliferation *in vitro* (5,7). Similarly, TTFields showed the greatest inhibitory effect at up to 150 kHz in non-small cell lung cancer *in vitro* (26). Clinical studies are currently underway for the use of TTFields in the treatment of brain metastases from lung (150 kHz), non-small cell lung (150 kHz), ovarian (200 kHz) and pancreatic (150 kHz) cancer, as well as mesothelioma (150 kHz) (27,28). Based on this previous work, the TTFields used in the present study were set at 150 kHz to inhibit the growth of liver cancer cell lines *in vitro*, and the results provided evidence supporting the potential use of TTFields treatment in liver cancer.

HCC is associated with multiple genetic aberrations, demonstrating the involvement of various signaling pathways in its initiation and progression. Patients suffering from HCC at advanced stages or those who have unresectable tumors typically receive treatment with sorafenib, which is a multi-kinase inhibitor (29). However, sorafenib can only improve the survival of patients by ~3 months (30,31), indicating that monotherapy is not sufficient to improve the outcome of patients with HCC. Therefore, a combination therapy that can simultaneously target multiple pathways to prevent the invasion and proliferation of HCC is urgently needed.

The present study demonstrated that TTFields induced apoptosis *in vitro*, which should be further explored in a xenograft model *in vivo*. Sorafenib treatment was reported to decrease the expression levels of phosphorylated (p)-Akt, PI3K, p-mTOR and p-p70S6K, and to inhibit the PI3K/Akt/mTOR signaling pathway in HCC cells (32,33). The present results demonstrated that TTFields and sorafenib combination treatment could inhibit liver cancer cell proliferation and invasion, suggesting that this treatment could prevent metastasis by synergistically enhancing apoptosis. As an alternative approach to inhibit tumor growth, it is important to establish whether TTFields-induced autophagy is related to the viability of cancer cells or to their sensitivity to apoptosis.

Moreover, undiscovered and potentially confounding synergistic properties may be present with a multitude of other novel or repurposed agents. This is evident through preliminary reports of TTFields with bevacizumab, as well as TTFields combined with trifluoropromazine, an approved antipsychotic drug (34). Trifluoropromazine has been identified to inhibit mitotic slippage when used in combination with TTFields (34,35). This is particularly notable, as the treatment appeared to decrease cell counts by up to 14% when used in combination, thus suggesting improved efficacy for the treatment of liver cancer (36). Cells receiving the combined treatment increased in size by up to 35%, suggesting decreased clonogenic potential in these cells (37). TTFields have also been combined with withaferin A, a steroidal lactone originating from the winter cherry plant, *Withania somnifera*. Additionally, withaferin A has been previously shown to have efficacy against glioma cell lines *in vitro* and in murine orthotopic GBM models (38). As seen in other combinational therapeutic strategies with TTFields, greater efficacy was achieved in treating liver cancer and other types of cancer when combining TTFields with withaferin A (39). The anti-microtubular class of mitotic inhibitors can be divided into microtubular-stabilizing and microtubular-destabilizing agents. In combination with TTFields, these agents have demonstrated antitumor activity in a variety of cancer types, such as liver, breast and ovarian cancer (40).

In conclusion, the present findings suggest that the combination of TTFields and other agents, especially sorafenib, promotes the apoptosis while inhibiting the proliferation and invasion of liver cancer cells *in vitro*. These results offer a potential strategy for using this combination chemotherapeutic treatment in patients with liver cancer in a clinical setting.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

YJ, WSL and EHK designed the study, the experimental setup and wrote the manuscript. YJ, WSL, SS, JYK and JKK performed the experiments. YJ, WSL, EHK, JYK and JKK analyzed and confirmed the data. YJ, WSL and EHK confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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