Peer Review File

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Reviewer A

The Authors investigated in vitro effect and mechanisms of a combination of TTFs and Sorafenib in two CRC cell lines. The authors concluded: (1) Combination treatment inhibited cell proliferation and induced apoptosis via ROS generation, (2) combination treatment with TTFields and sorafenib inactivates AKT/STAT3 signaling pathway, and (3) combination treatment with TTFields and sorafenib is clinically applicable for treating metastatic CRC.

The same group previously reported TTFs induced cancer cell (GBM) death through the inhibition of STAT3. Akt pathway has been widely studied in the Sorafenib induced cell death. The improvement of in vitro cell death and inactivation of STAT3/AKT pathways is expected when the combination treatment is carried out.

ROS was induced due to the combination treatment and single treatment. However, this did not necessarily mean the cell death was induced via ROS, which may just be a collateral product or the result of cell damage. It needs more strict experiments to demonstrate the ROS product at this level can induce/contribute to cell death.

The safety and efficacy of combination treatment with TTFields and sorafenib have not been studied in vivo in animal models yet. Without any experimental and clinical evidence, to claim its clinical application was not scientifically sound.

There are several major issues need to be addressed.

1, There are many English typos and grammatical errors. Some examples are: (a) 5capecitabine,-fluorouracil (5-FU)/LV, (Lines 50-51); (b) additting the targeted therapy (Line 80); (c) TTFields was produced (Line 91); (d) To treat the electric field to cell lines (Lines 92/93); (e) TTFields was added (Line 116)… There are many more.

Answer) We are so sorry for our errors and have revised them all.

2, Introduction did not mention current advance, known mechanisms of single modality.

Answer) As you requested, we have added these points and references.

3, “Materials and Methods” lack of details and necessary information. For example, (a) “antibiotics” (Line 104) are what antibiotics and concentration? (b) “The cell viabilities were then measured using a Multiskan EX Microplate Photometer (Thermo Fisher Scientific) at 450 nm.” (Lines 112-113) How are OD values at 450nm associated with cell viability? (c) “Colony-Forming Assay” (115-118) did not specify what cell concentrations, agarose gel used or not, etc.?

Answer) We are so sorry for our errors and have revised them all.

a) We have added the information of antibiotics.

b) We followed the protocol of this product for detecting the cell viability. The instruction

recommends as like this sentence;
We recommend using 450nm. If there is no 450nm filter, the wavelength between 420-480nm measurement is possible

4, In Statistical analysis, t-test was used for all data analysis. However, most data include more than two groups or treatments. One-Way ANOVA is more appropriate.
Answer) We did Statistical analysis again using One-Way ANOVA.

5, Signal pathway study designs did not include positive controls or pathway inducers and negative controls or pathway blockers to confirm the role of respective pathways actually involved in the cell death.
Answer) As you requested, we did the experiment with pathway inhibitors
(LY294002: Akt pathway inhibitor, Stattic: Stat3 inhibitor).

Result) When treated with TTFields+Sorafenib and LY294002 or Stattic, a selective Akt pathway inhibitor and Stat3 inhibitor, an additive inhibitory effect on Akt and STAT3 pathway was observed in HCT 116 cells. The protein levels of p-Akt or p-STAT3 in LY294002 or Stattic co-treated cells were much more reduced compared with that in inhibitor alone treated cells (Fig. 4a).

6, To claim the application of this combination treatment for metastasis is overstated without any results from in vitro cell migration and in vivo metastatic models.
Answer) We are so sorry for error and removed this sentence.

Reviewer B

The article entitled "Tumor-treating Fields in combination with Sorafenib curtails the growth of colon cancer by inactivating AKT / STAT3 signaling" is of great interest, well written, and results well presented.
However, some modifications would be necessary:
- In the methodology section, it would be convenient to detail the information in more detail. For example, the concentration of the compounds with which the medium is supplemented (L-glutamine...).
- It is necessary to unify the numbering since in some sections it appears as 5000 and in others as 5x10^4.
Answer) As your request, we have revised the methodology and added the reference in the revised manuscript.

Cell culture
Human HCT116 and SW480 colorectal cancer cell lines (CRC) were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA). HCT116 and SW480 cells were grown in DMEM supplemented with 10% heat-inactivated FBS (both Gibco; Thermo Fisher Scientific, Inc.), 0.1 mM non-essential amino acids, glutamine, HEPES and penicillin

(100Uml−1100 U ml−1) at 37°C in a 5% CO2 humidified incubator.

Cell-Viability Assay

Cells were set at a confluency of 5 × 103 5,000 cells per well in a 96-well plate and incubated for 24 h.

Colony-Forming Assay

TTFields were added to cells 6 h after sorafenib treatment to a last concentration of 3 μmol/L, and the cells were then maintained for 48 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 (8).