Poster 165

Preclinical Assessment of Treatment Scheduling for Tumor Treating Fields (TTFields) Applied Concurrently with Radiation Therapy in Glioblastoma

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Introduction

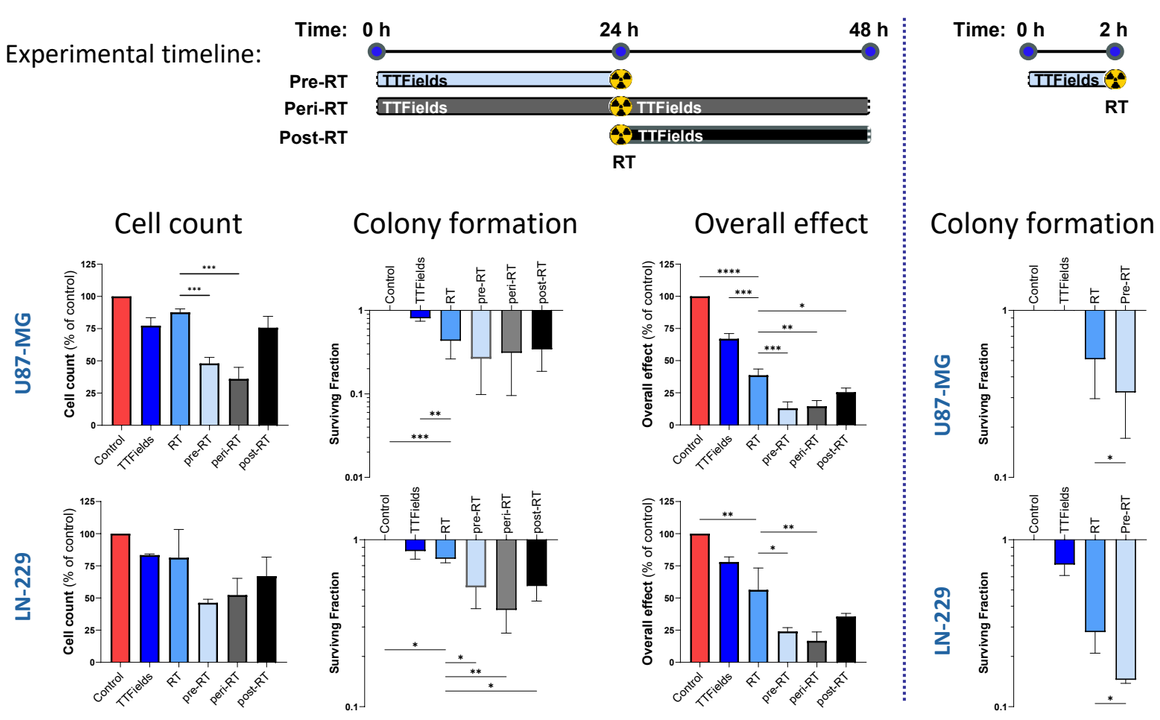
- Tumor Treating Fields (TTFields) are electric fields that exert physical forces to disrupt cellular processes critical for cancer cell viability and tumor progression.[1,2]
- TTFields therapy is approved for treatment of newly diagnosed and recurrent glioblastoma (GBM), unresectable pleural mesothelioma, metastatic non-small cell lung carcinoma (NSCLC), and locally advanced pancreatic cancer.[3-8]
- While prolonged TTFields exposure is known to impair DNA repair mechanisms, the impact of brief TTFields application has not been investigated.[9-11]
- This study investigated whether the timing and sequencing of TTFields relative to radiation therapy (RT) enhance radiation-induced DNA damage in GBM.

Methods

- **In vitro experiments:** Human GBM cell lines (U87-MG, LN-229, U118-MG) were left untreated or exposed to TTFields (200 kHz, 1.7 V/cm RMS) using the inovitro system and/or to RT (2 Gy) using the X-Rad320 X-ray irradiator, according to the scheduling below.
- **Cell count, clonogenicity and overall effect:** Following 48 h treatment, cells were counted by flow cytometry. Then, cells were harvested, re-plated, and grown for 14-21 days. Colonies were stained with crystal violet and counted, and the surviving fraction calculated. Overall effect was calculated by multiplying colonies with respective ell count.
- **DNA damage in vitro:** Following 48 h treatment, cells were fixed, permeabilized, stained with Zombie NIR and anti-ɣH2AX antibody, and examined by flow cytometry.
- **Gene/protein expression analysis:** Cells were treated with TTFields (200 kHz, 1.7 V/cm RMS) for 2 h. RNA extracts were analyzed using an RT² Profiler PCR Array targeting 82 DNA damage response genes. Protein lysates were examined by Western Blot for Fanconi anemia-BRCA pathway proteins.
- **In vivo experiment:** C57BL/6 mice were intracranially injected with murine GL261-mCherry glioma cells. 48 h-treatment started on day 18 post-injection, with TTFields (200 kHz) and RT (6 Gy) applied according to the scheduling below.
- **Single cell suspension analysis:** At study end, tumors were processed into single cell suspensions, fixed, permeabilized, and stained for ɣH2AX, Ki-67, CD45, and Zombie NIR. mCherry gating was used for identifying tumor cells.

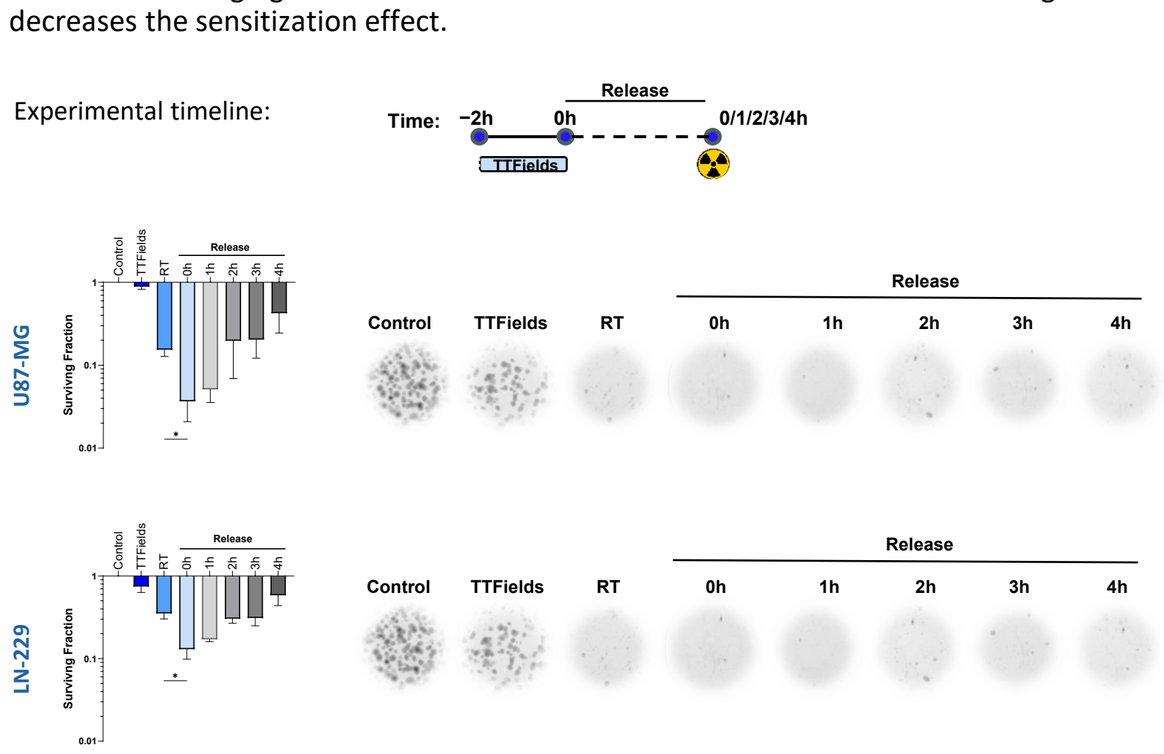
Results

FIGURE 1. TTFields exposure sensitized U87-MG cells to RT.



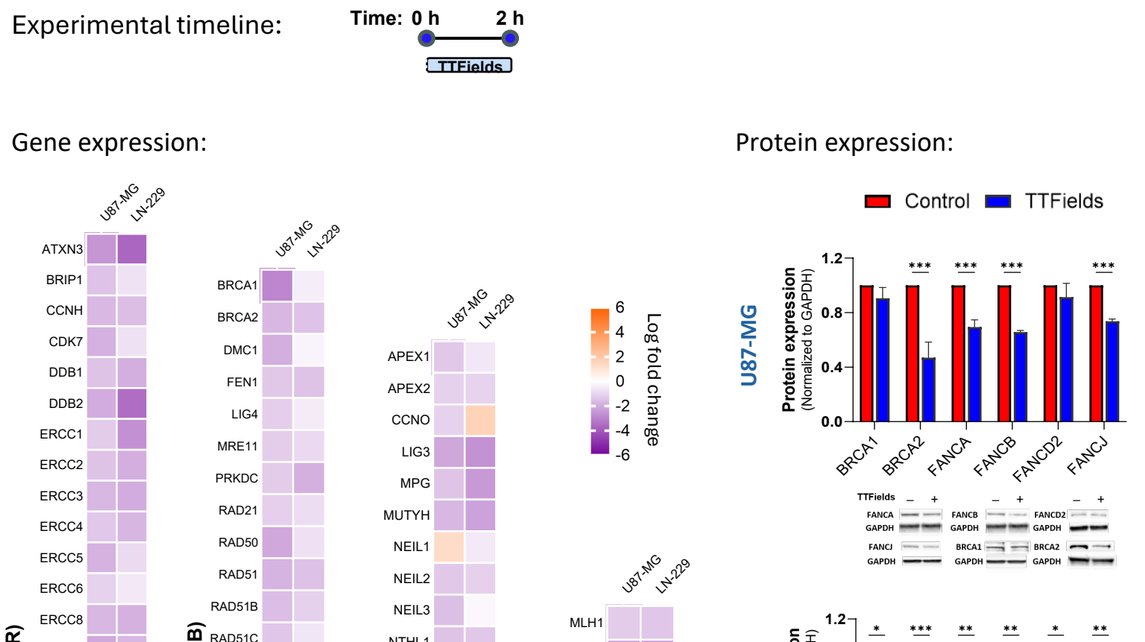
One-way ANOVA with Tukey's multiple comparison ;*p < 0.05, **p < 0.01, ***p < 0.001

FIGURE 3. Prolonging the time interval between TTFields cessation and RT dosing decreases the sensitization effect.



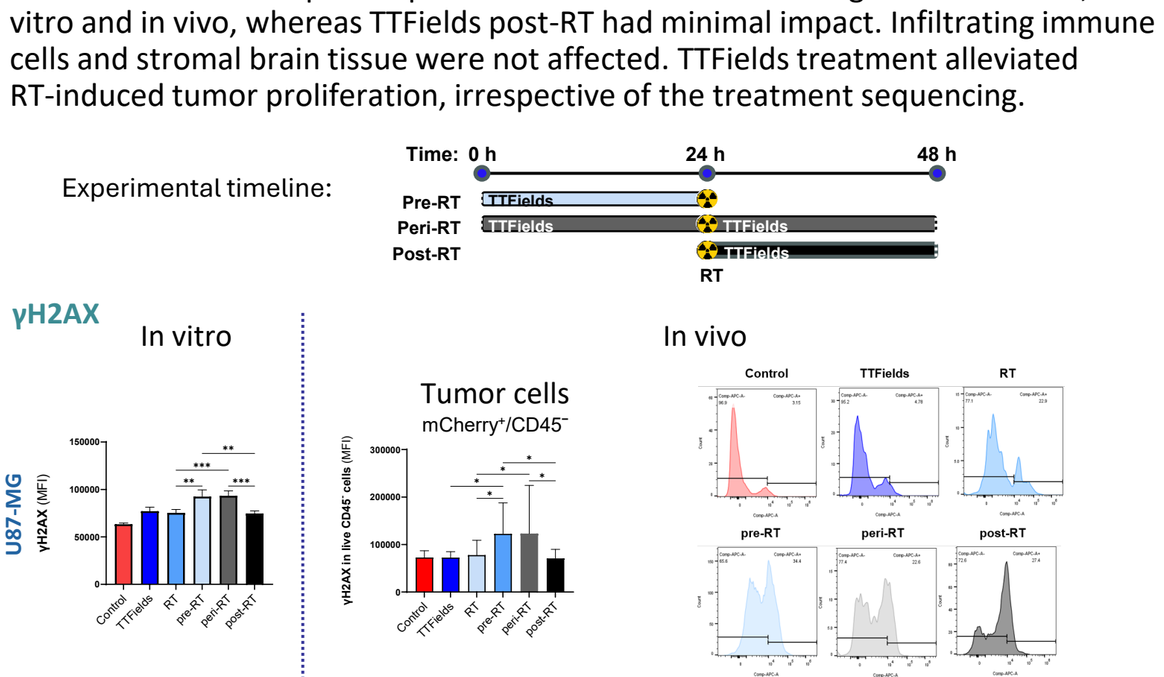
One-way ANOVA with Tukey's multiple comparison ;*p < 0.05

FIGURE 2. Brief TTFields exposure downregulated DNA damage response genes.



Multiple t-test; *p < 0.05, **p < 0.01, ***p < 0.001

FIGURE 4. TTFields exposure prior to RT increased DNA damage in cancer cells, in vitro and in vivo, whereas TTFields post-RT had minimal impact. Infiltrating immune cells and stromal brain tissue were not affected. TTFields treatment alleviated RT-induced tumor proliferation, irrespective of the treatment sequencing.



One-way ANOVA with Tukey's multiple comparison ;*p < 0.05

Conclusions

- These preclinical results underscore the significance of optimizing the timing and sequence of TTFields therapy to enhance radiation-induced DNA damage in GBM.
- TTFields-induced sensitization of GBM to RT may be linked to downregulation of DNA damage response pathways.

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