# Short Exposure to Tumor Treating Fields (TTFields) Induces DNA Repair Pathway Downregulation and Radiosensitization in Glioblastoma Cells

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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, and metastatic non-small cell lung carcinoma (NSCLC).[3-7]
- While prolonged TTFields exposure is known to impair DNA repair mechanisms, the impact of brief TTFields application has not been investigated.[8-10]
- Here, we investigated whether short exposure to TTFields sensitizes GBM cells to radiation therapy (RT) by downregulating key DNA repair pathways.

## Methods

- **TTFields/RT in vitro experiments:** Human GBM cell lines (U87-MG, LN-229, U118-MG) were cultured under standard conditions. Cells were seeded at $1 \times 10^5$ cells/well in inovitro dishes and exposed for 2 h to TTFields (200 kHz, 1.7 V/cm RMS) using the inovitro system. Control groups were maintained under identical conditions without TTFields. TTFields-treated and control cells were then exposed to RT (2 Gy) using an X-Rad320 X-ray irradiator, or left untreated.
- **Clonogenic survival assay:** Treated cells were harvested, re-plated, and grown for an additional 14-21 days. Colonies were stained (0.5% crystal violet solution), counted, and clonogenic effect was calculated relative to control.
- **TTFields/RT in vivo experiments:** C57BL/6 mice were intracranially injected with murine GL261-mCherry glioma cells. On day 17 post-injection, tumors were imaged by MRI. Treatment started the next day. Mice were randomly assigned to six groups (N=4–6 per group):
  1. Heat only (24 h)
  2. TTFields only (200 kHz, 24 h)
  3. Heat (20 h) followed by RT (6 Gy), then Heat (4 h)
  4. Heat (20 h) followed by RT (6 Gy), then Heat (20 h)
  5. TTFields (200 kHz, 20 h), RT (6 Gy), Heat (20 h)
  6. TTFields (200 kHz, 20 h), RT (6 Gy), TTFields (200 kHz, 20 h)
- **DNA damage measurement:** Tumor single cells suspensions were prepared from the tumors, and analyzed by flow cytometry to assess γH2AX levels.
- **Gene/protein expression analysis:** RNA extracts were analyzed with a PCR array for DNA damage response genes. Protein lysates were examined by Western Blot for Fanconi anemia-BRCA pathway proteins.

## Conclusions

- Brief exposure to TTFields effectively suppressed DNA repair mechanisms and increased the sensitivity of GBM cells to RT.
- Increased tumor DNA damage was observed, with the most pronounced effect occurring when TTFields were administered again following RT.
- These preclinical results underscore the significance of optimizing the timing and sequence of TTFields therapy to enhance radiation-induced DNA damage in GBM. This approach is being further evaluated in the ongoing Phase 3 TRIDENT clinical trial (NCT04471844).

**References: 1.** Kirson ED, et al. Cancer Res, 2004. 64(9):3288-95; **2.** Kirson ED, et al. Proc Natl Acad Sci USA, 2007. 104(24):10152-7; **3.** Stupp R, et al. Eur J Cancer, 2012. 48(14):2192-202; **4.** Stupp R, et al. JAMA, 2015. 314(23): 2535-43; **5.** Stupp R., et al. JAMA, 2017. 318(23):2306-16; **6.** Ceresoli GL, et al. Lancet Oncol, 2019. 20(12):1702-9. **7.** Leal T, et al. Lancet Oncol, 2023. 24(9): 1002-1017. **8.** Karanam NK, et al. Cell Death Dis, 2017. 8(3): e2711. **9.** Mumblat H, et al. Lung Cancer, 2021. 160: 99-110. **10.** Berckmans Y. et al. Front Oncol, 2024. 14:1402851.

## Results

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

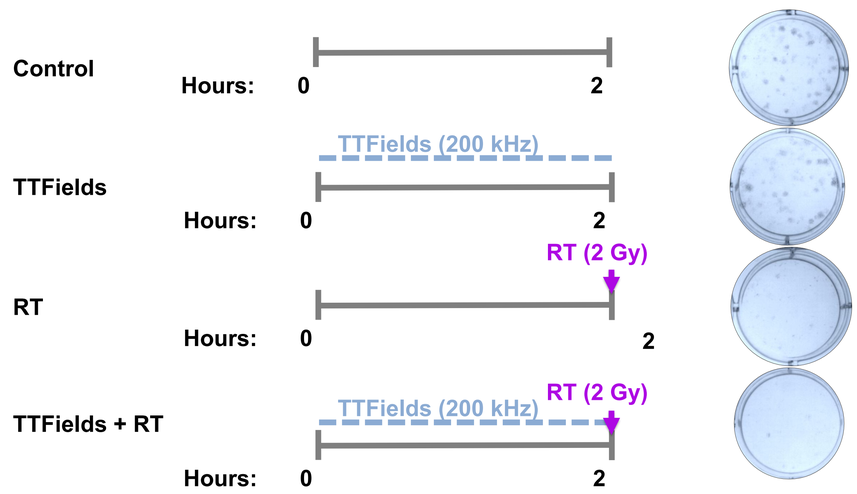


**FIGURE 2.** Short exposure of U87-MG cells to TTFields (200 kHz, 2 h) downregulated DNA damage response genes.

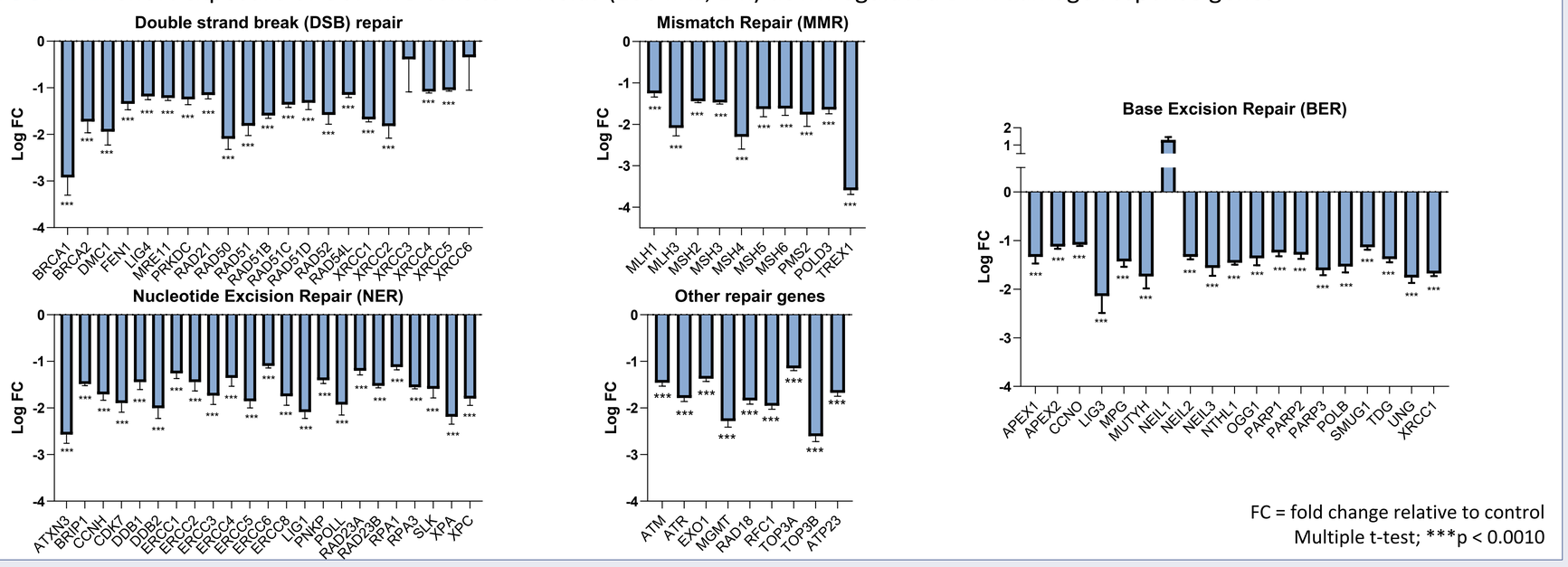


**FIGURE 3.** Short exposure to TTFields (200 kHz, 2 h) induced downregulation of DNA repair proteins across GBM cells.

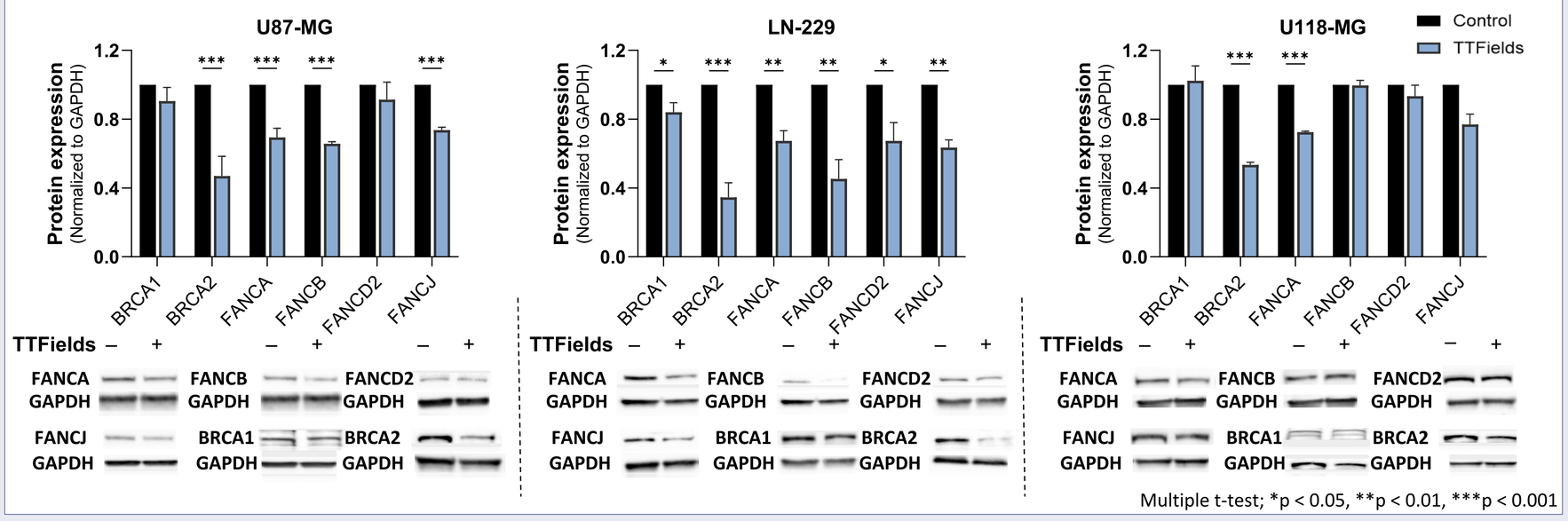


**FIGURE 4.** Exposure to RT in mice increased DNA damage and reduced the expression of DNA repair proteins not only in tumor cells but also in tumor-infiltrating immune cells, whereas TTFields treatment did not significantly alter DNA damage levels in either cell type.

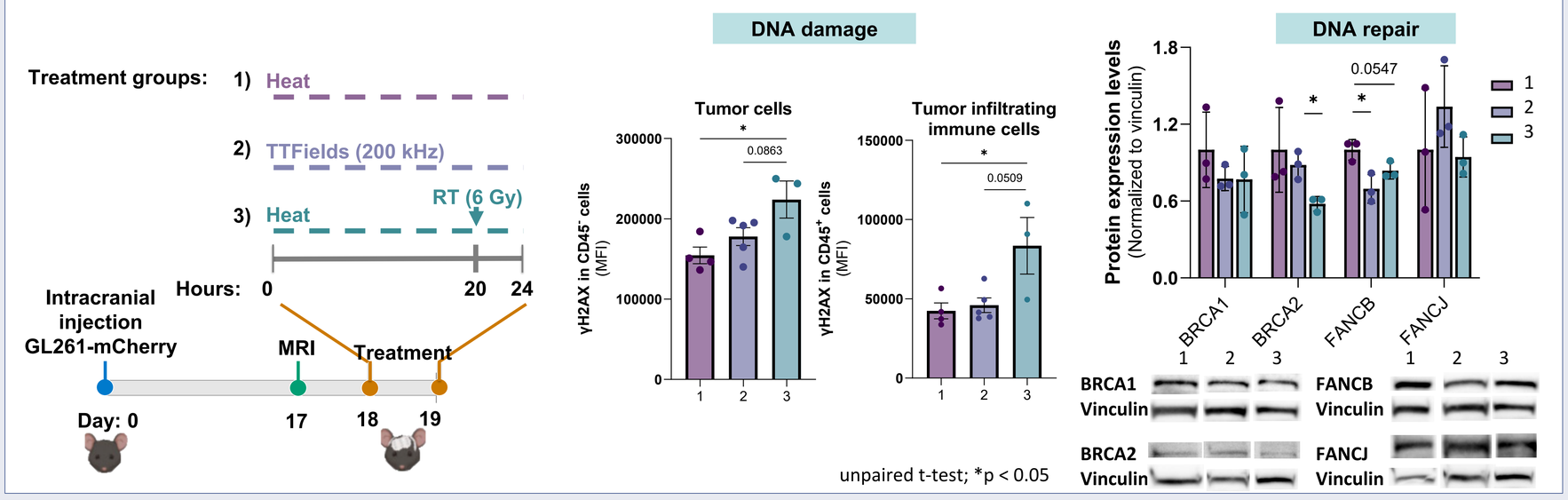


**FIGURE 5.** Exposure to RT in mice that were primed with TTFields resulted in increased DNA damage and a reduction in DNA repair protein levels within tumor cells. This effect was more pronounced when TTFields treatment was continued after RT compared to when TTFields were discontinued following RT. Immune cells showed minimal changes in response to these treatments.