Tumor Treating Fields (TTFields) induce pro-inflammatory phenotype skewing of macrophages

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Introduction

- Tumor Treating Fields (TTFields) are electric fields that exert forces to disrupt cellular processes critical for cancer cell viability and tumor progression [1,2]
- TTFields exert directional forces on polar tubulins leading to reorganization of the microtubule network, resulting in activation of GEF-H1/RhoA/ROCK signaling[3]
- GEF-H1 signaling is required for activation of dendritic cells[4] and is involved in polarization of macrophages between their pro- and anti-inflammatory phenotypes (M1 and M2, respectively)[5]
- We have previously shown that TTFields-induced cancer cell death stimulates anti-tumor immunity and promotes the maturation of dendritic cells[6]
- In the current study, we investigated TTFields potential to directly regulate macrophage phenotype skewing

Methods

- Bone marrow cells were flushed from the femurs and tibias of 5-8 week old C57BL/6 mice to generate bone marrow–derived macrophages (BMDMs) and were differentiated for 7 days in GM-CS/F
- BMDMs were stimulated for 24 h with IFN-γ with or without LPS (M1 polarization) or IL-4 (M2 polarization)
- Cells were treated with TTFields (1.75 V/cm RMS, 150 kHz) for 24 h or for shorter durations as indicated using the inovitro™ system
- Flow cytometry was used to assess the expression of F4/80 (pan macrophages), and the activation markers MHC II, CD80, iNOS, CD206, and Arg-1. Viability 405/452 or Zombie NIR fixable dyes were used for the discrimination of dead cells
- A multiplexed secretion assay was used to measure the heterogeneity of the stimulated macrophages. We captured the secretion of 13 different proteins including CXCL1 (KC), IL-18, IL-23, IL-12p70, IL6, TNF-α, IL-12p40, free active TGF-β1, CCL22 (MDC), IL-10, IL-6, G-CSF, CCL17 (TARC) and IL-1β. Cytokine secretion levels were normalized to control
- Cell lysates were prepared, and total and phosphorylated GEF-H1, c-Jun, and p65 were measured using Western blot. Band intensities were quantified relative to GAPDH, and expressed as the ratio relative to cells at time zero
- Cell lysates were prepared, and total and activated RhoA were measured using a G-LISA activation assay and an ELISA assay, respectively, according to the manufacturer's instructions. RhoA activity relative to total RhoA was expressed as the ratio relative to cells at time zero

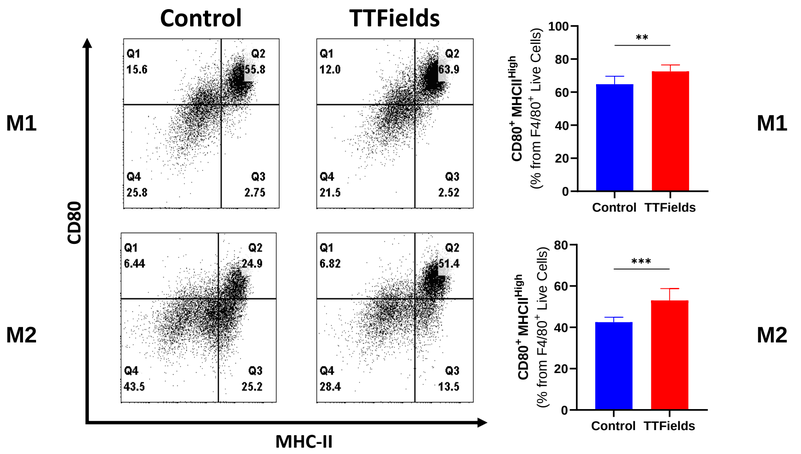
Conclusions

- This study revealed a novel immunomodulatory role for TTFields, promoting in vitro pro-inflammatory macrophage polarization and activation.
- TTFields exhibited LPS-like activity, serving as a second signal to polarize M1 macrophages possibly via transcriptional response driven by the GEF-H1/RhoA/ROCK/NFκB pathway.

References: 1. Kirson, E. D., et al. (2004) Cancer Res **64**(9): 3288-3295. 2. Kirson, E. D., et al. (2007) Proc Natl Acad Sci U S A **104**(24): 10152-10157. 3. Voloshin, T., et al. (2020). Cancers **12**(10): 3016. 4. Kashyap, A. S., et al. (2019). Cell Reports **28**(13): 3367-3380.e3368. 5. Roser, A.-E., et al. (2017). Frontiers in aging neuroscience **9**: 94-94. 6. Voloshin, T., et al. (2020). Cancer Immunol Immunother **69**(7): 1191-1204.

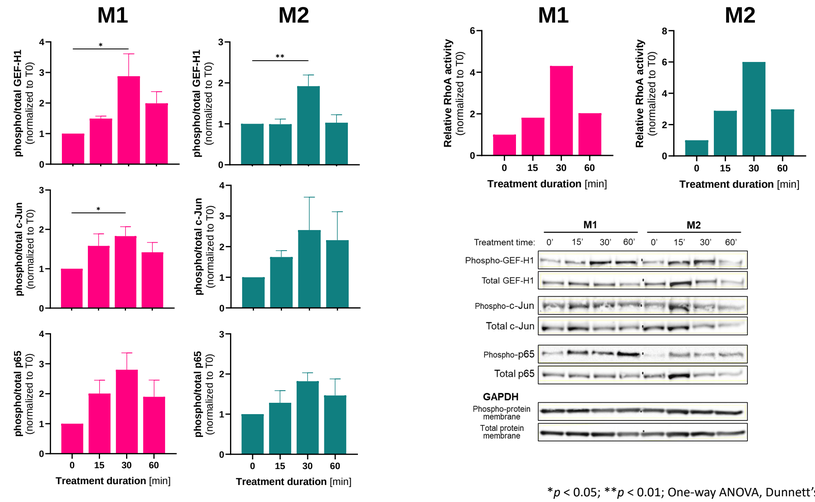
Results

Figure 1: TTFields polarized M2 macrophages to the pro-inflammatory M1 phenotype and elevated the pro-inflammatory nature of M1 macrophages, as indicated by increased CD80 and MHC-II expression



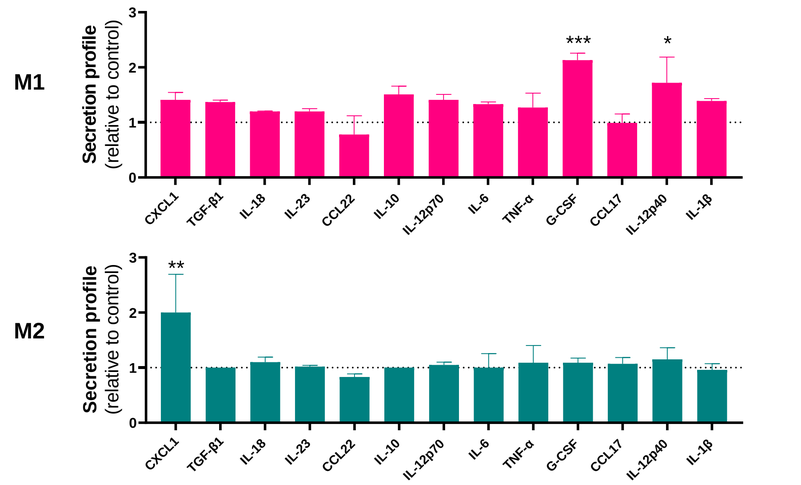
**p < 0.01; ***p < 0.001; unpaired t-test

Figure 2: TTFields activated M1 inflammatory signaling via p65 and GEF-H1



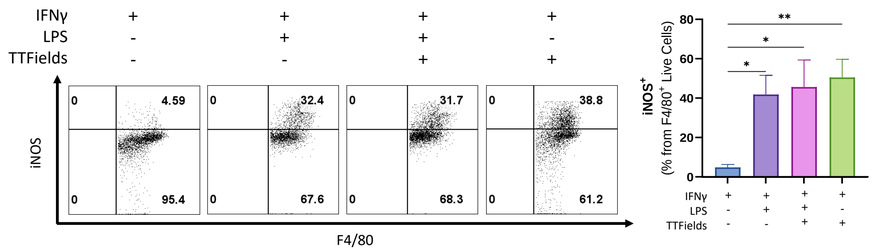
*p < 0.05; **p < 0.01; One-way ANOVA, Dunnett's test

Figure 3: TTFields promoted secretion of CXCL1 by M2 macrophages, and reinforced the pre-existing M1 inflammatory phenotype



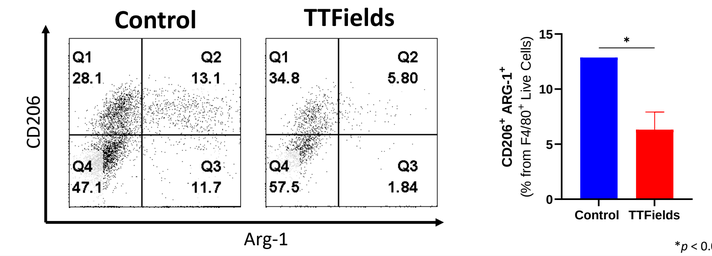
*p < 0.05; **p < 0.01; ***p < 0.001; Two-way ANOVA, Sidak's test

Figure 4: TTFields elevated iNOS expression in IFNγ-treated macrophages similarly to LPS



*p < 0.05; **p < 0.01; One-way ANOVA, Dunnett's test

Figure 5: TTFields diminished the Arg-1+ population of M2 macrophages



*p < 0.05; unpaired t-test

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