TUMOR TREATING FIELDS (TTFIELDS) APPLICATION INDUCES A PRO-INFLAMMATORY PHENOTYPE IN MACROPHAGES


Background Tumor Treating Fields (TTFields) are electric fields that disrupt cellular processes critical for cancer cell viability and tumor progression. Cancer cell death following application of TTFields has previously been shown to be immunogenic and promote dendritic cell maturation. In the current research, we investigated possible effects of TTFields on macrophage phenotypic regulation.

Methods Bone marrow-derived macrophages (BMDMs) were generated from the femurs and tibias of 5–8-week-old Balb/C mice. The BMDMs were then stimulated with LPS+IFN-γ (M1 polarization) or IL-4 (M2 polarization), and treated with TTFields (150 kHz) for 24 h. The cells were then examined by flow cytometry for surface expression of the macrophage biomarker F4/80 and the activation markers CD80, major histocompatibility complex class II (MHC II), inducible nitric oxide synthase (iNOS), CD206, and ARG-1. Multiplexed secretion assays were employed on cell supernatants to measure secretion levels of CXCL1 (KC), IL-18, IL-23, IL-12p70, IL6, TNF-α, IL-12p40, CCL22 (MDC), IL-10, IL-6, G-CSF, CCL17 (TARC), and IL-1β. Cell lysates were examined with a RhoA activation kit; and by Western blot to determine phosphorylation levels of GEF-H1, c-Jun, and p65.

Results TTFields application increased the percentage of M1 and M2 BMDMs expressing the pro-inflammatory M1 markers CD80+ and MHC IIhigh, and decreased expression of the M2 markers CD206 and ARG-1. Cell supernatants demonstrated a pro-inflammatory secretion pattern, with increased levels of CXCL1, IL-18, IL-23, IL-12p70, IL6, TNF-α, IL-12p40, CCL22, G-CSF, CCL17 and IL-1β. TTFields application resulted in GEF-H1 and RhoA activation in both M1 and M2 BMDMs, a pathway previously shown to be activated following TTFields application, with subsequent activation of the transcription factors c-Jun and p65.

Conclusions TTFields treatment displays a novel immunomodulatory role, promoting macrophage pro-inflammatory polarization in vitro. This phenotypic skewing seems to involve activation of the GEF-H1/RhoA/ROCK pathway.

REFERENCES


Ethics Approval The work in this study was conducted in accordance with relevant laws and regulations and approved by the Novocure ethics board (approval number: NPC-No-IL-2111–103-1)

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