

The anti-proliferative effects of amplitude-modulated RF EMF (AM RF EMF) at tumor-specific frequencies on glioblastoma cells

Jimenez H, Herpai DM, Wang M, Sharma S, Matsuo K, Zhou, X, Lesser G, Debinski W, *Costa FP, **Barbault A, Blackman CF and Pasche B.

Department of Cancer Biology, Wake Forest School of Medicine and Comprehensive Cancer Center at Wake Forest Baptist Medical Center, USA; *Hospital Sírío Libanês, São Paulo, Brazil; **TheraBionic GmbH, Ettlingen, Germany

Introduction

Whole body administration of low-level radiofrequency electromagnetic fields, amplitude-modulated (AM RF EMF) at specific frequencies ranging from 181 Hz to 21 kHz showed efficacy in advanced breast cancer and hepatocellular carcinoma (HCC) (Barbault, Costa *et al.*, 2009, Costa de Oliveira *et al.*, 2011). *In vitro* studies demonstrates that AM RF EMF treatment inhibits cancer cell growth in a tumor-specific manner (Zimmerman, Pennison *et al.*, 2012). Indeed, the growth of both HCC (HepG2 and Huh7) and breast cancer (MCF-7) cells was inhibited when exposed to corresponding tumor-specific frequencies, 3 hours per day for 7 days. This work has led us to investigate AM RF EMF's potential impact on Glioblastoma (GBM).

Methods

The GBM cell line U-251, as well as two low passage GBM explant cells (BTCOE-4536 and BTCOE-4795), were exposed to 27.12 MHz RF EMF modulated at GBM-specific frequencies using exposure systems designed to replicate treatment at human exposure levels. Control cells were not exposed to any EMF. GBM cells were cultured for seven days exposed to GBM-specific frequencies for three hours daily. The specific absorption rate (SAR) of AM RF EMF was 0.4 W/kg, which is identical to the highest SAR measured in patients receiving treatment with this therapy. Proliferation was quantified via the tritiated thymidine incorporation assay. Neurosphere-formation was counted at 7 days post treatment. Statistics were performed by student's T-test. RNA-sequencing was performed by the Human Genomics shared facilities and resources.

Results

The growth of U-251, BTCOE 4536 and BTCOE 4795 cell lines was decreased by 19% (N = 10; p = 0.0018), 23% (N = 4; p = 0.0348) and 14.5% (N = 10; p = 0.0431) following exposure to GBM-specific AM RF EMF. Neurosphere-forming ability of the U-251 and BTCOE-4795 cell lines was inhibited by 48.0% (N = 6; p = 0.0040) and 31.7% (N = 6; p = 0.0002) following exposure to GBM-specific AM RF EMF. RNA-seq identified the Mitotic Roles of Polo-Like Kinase pathway as being affected by GBM-specific AM RF EMF treatment.

Mitotic Roles of Polo-Like Kinase	
Gene	Fold Change
Abnormal Spindle Microtubule Assembly (ASPM)	1.68 (increase)
Polo-Like Kinase 1 (PLK-1)	1.89 (increase)
Polo-Like Kinase 4 (PLK-4)	1.61 (increase)
Centrosomal Protein 152 (CEP152)	1.18 (increase)
Cyclin B1 (CCNB1)	1.72 (increase)

Table 1. Mitotic Roles of Polo-Like Kinase pathway. Pathway analysis of RNA-seq data from the U251 cell line identified this canonical pathway as being modulated by GBM-specific AM RF EMF. Fold change reported is from RNA-seq analysis

Results (cont.)

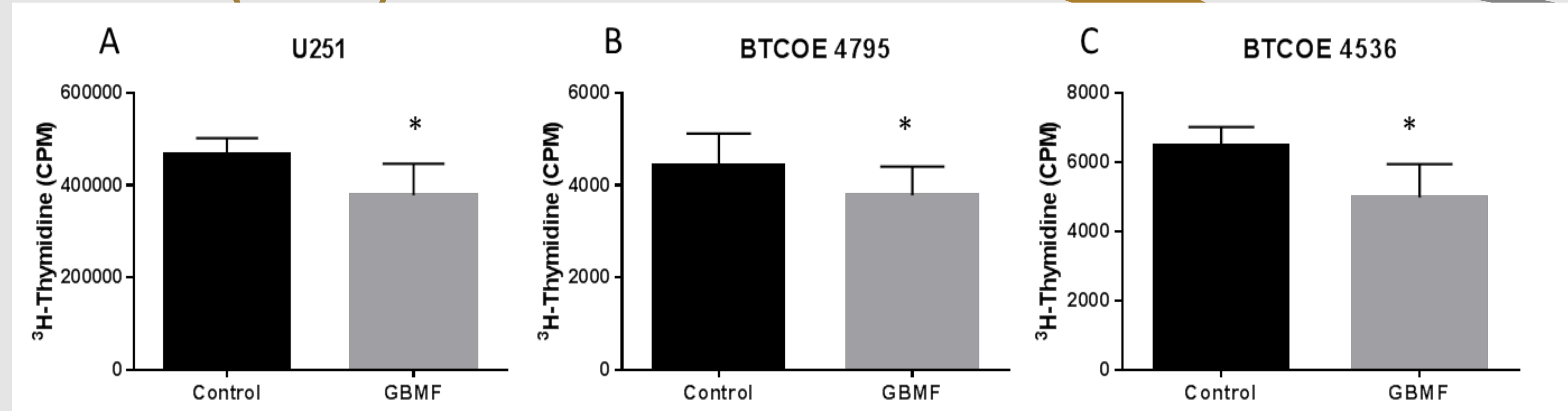


Figure 1. A-C Cell proliferation assay of cell lines exposed to GBM-specific modulation frequencies using tritium incorporation. A. The growth U251 cells were inhibited by 19% (N = 10; p = 0.0018). B. The growth BTCOE 4795 cells were inhibited by 14.5% (N = 10; p = 0.0431) C. The growth BTCOE 4536 cells were inhibited by 23% (N = 4; p = 0.0348).

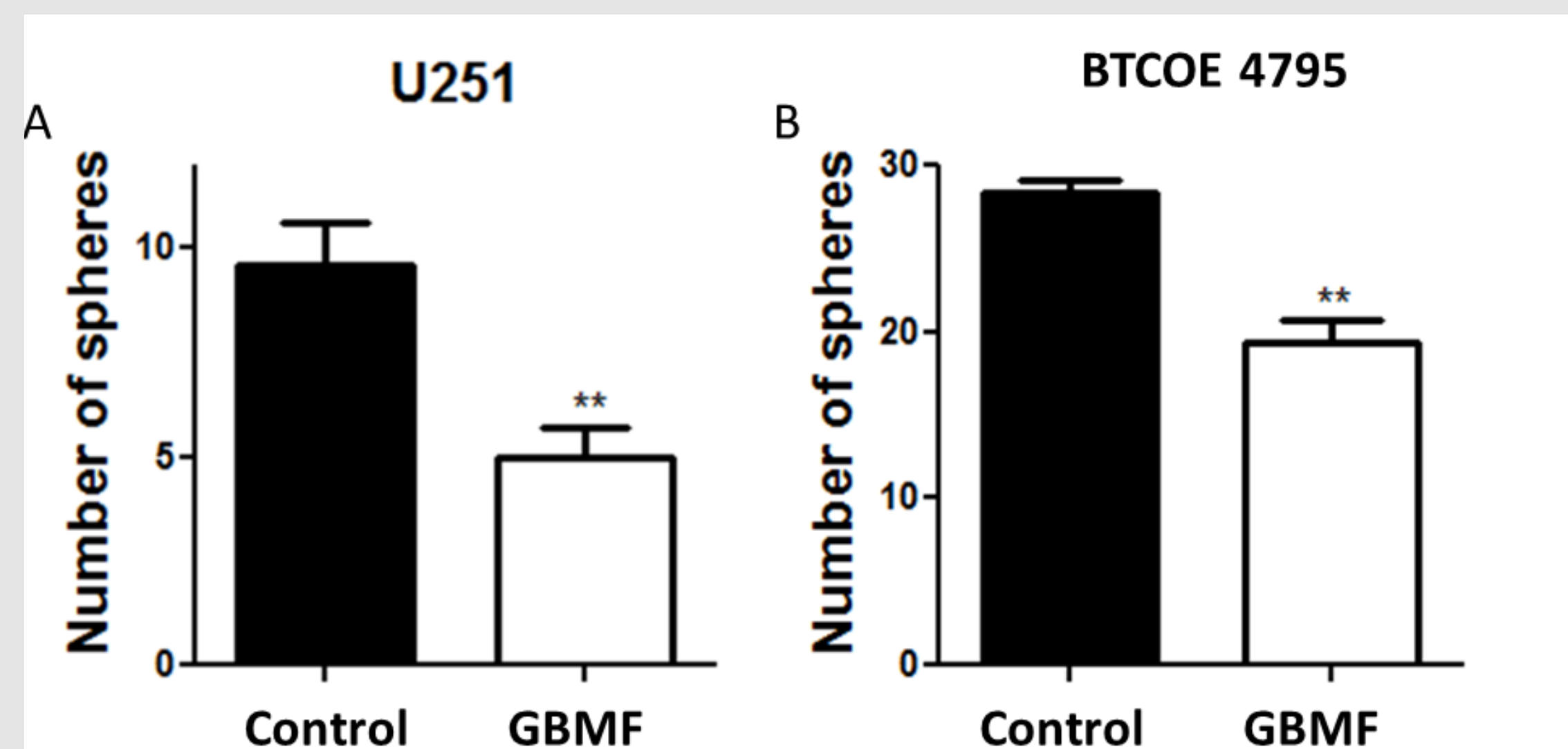


Figure 2. A & B Neurosphere formation assay. A. Neurosphere forming ability of U251 cells were inhibited by 48% (N = 6; p = 0.0040). B. Neurosphere forming ability of BTCOE 4795 cells were inhibited by 31.7% (N = 6; p = 0.0002).

Conclusions

- GBM-specific AM RF EMF inhibits proliferation of three different GBM cell lines (Fig 1 A-C)
 - U251, BTCOE 4795 and BTCOE 4536
- GBM-specific AM RF EMF inhibits the neurosphere forming ability of GBM cell (Fig 2 A & B)
 - U251 and BTCOE 4536
- RNA-seq of U251 cells highlights the Mitotic Roles of Polo-Like Kinase canonical pathway as the prime pathway impacted as a result of GBM-specific AM RF EMF
- GBM-specific AM RF EMF should be further studied as a new treatment modality of GBM.

Future experiments

- Intracranial U251 cell implantation xenograft is currently underway
- qRT-PCR validation of Mitotic Roles of Polo-Like Kinase pathway RNA-seq results
- Knockdown of PLK-4 and CEP152 for in GBM cells for exposure functional assay
- Flow cytometry for stemness markers to validate neurosphere formation data
- Mitotic spindle visualization and quantification in GBM-specific treated cells

References

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Contact Information

E-mail: hjimenez@wakehealth.edu