PTGER3 regulates Tumor Treating Fields resistance in glioblastoma cells

Michelle Nguyen, Dongjiang Chen, Adam O'Dell, David Tran

Tran Lab, Dept of Neurological Surgery, Bridge Institute, University of Southern California, Los Angeles, CA , USA

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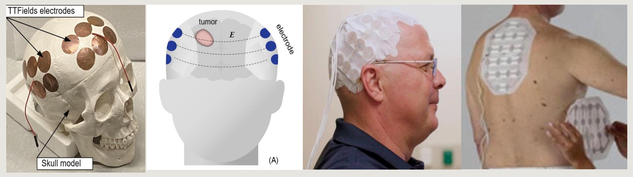
USC University of Southern California

Abstract

Glioblastoma Multiforme (GBM) stands as the primary deadliest brain cancer, with more than 13,400 people in 2022 diagnosed with the disease in the US alone. With temozolomide (TMZ) as the only chemotherapy treatment, diagnosed patients have a progression-free survival (PFS) span of 15 months. However, a novel treatment, Tumor Treating Fields (TTF), uses noninvasive, low intensity alternating electric fields to rupture the cell's nuclear envelope and hinder the mitotic growth by stimulating cell death. This cancer treatment has been clinically proven to be a successful way to counter multiple cancer types and increase GBM PFS to 21 months. Unfortunately, a recent discovery shows that close to 100% of patients given TTFields developed a resistance in treated cells that decreases efficacy of the treatment. This has been characterized by alteration of the cell tumor microenvironment by translocation of prostaglandin E receptor 3 (PTGER3, or EP3), a Gai-protein-coupled cell surface receptor, to the nucleus to bind to zinc finger protein 488 (ZNF488), a transcription factor. Through computational algorithms and experimental procedures, we found that EP3 is rapidly upregulated when exposed to TTFields, leading to new resistance in sensitive GBM cells to the treatment. These results identify EP3 as a major factor in GBM cell resistance and establish the receptor as a potential target for enhancing therapeutic efficacy of TTFields. Furthermore, as patients are usually given TMZ for chemotherapy and TTFields as a secondary treatment, we explored combination of both treatments and found EP3 to increase in dual treatment. This finding will allow us to jumpstart our exploration of treatment combinations to ensure the best results for anti-cancer therapy.



Objectives

GBM cell developed resistance to TTFields leads us to focus on understanding the mechanisms of TTFields resistance in the immunity aspect. With the help of computational and experimental biology techniques, we can identify responsible regulating networks and potential targets of TTFields resistance and determine the efficacy of combining different anti-cancer. This way, we can understand how GBM cells develop resistance to the cytotoxic effects of TTFields over time and the effect of using TMZ and TTFields together to improve therapeutic efficacy of TTFields treatment.

Background

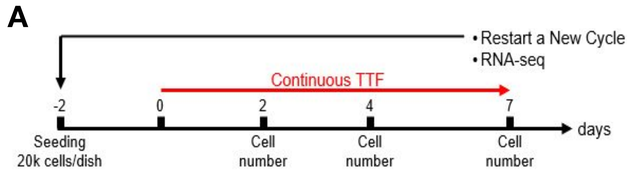
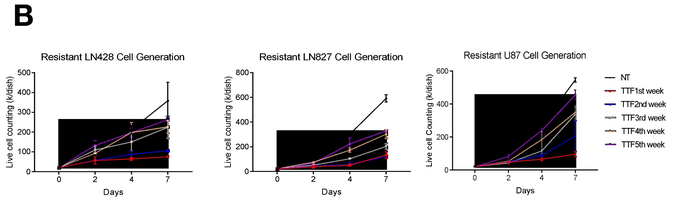


Figure 1: Generation and characterization of TTFields-resistant GBM cells. (A) Treated cells from 3 human GBM cell lines (LN428, LN827, and U87) with TTFields at a frequency of 200 kHz until TTFields-resistant cells were detected. We collected cells every 7 days of TTFields treatment with 2-day breaks in between replating dates for recovery before treatment resumed. **(B)** GBM cell lines are shown to gradually begin resisting TTFields treatment and eventually develop similarities in cell growth rates to the control groups not treated with TTFields in a span of 4-5 weeks.



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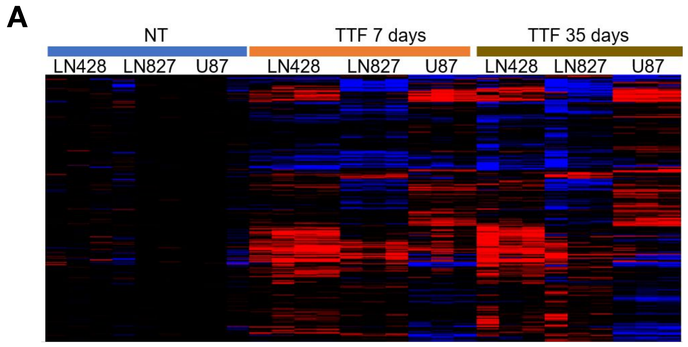


B

nSCORE Rank								
Gene	ALL NT vs 7d	ALL NT vs 35d	LN428 NT vs 7d	LN428 NT vs 35d	LN827 NT vs 7d	LN827 NT vs 35d	U87 NT vs 7d	U87 NT vs 35d
FLI1	73	1	50	25	2094	2094	2094	6
EHF	2	2	7	5	2094	199	150	30
ELF3	28	3	23	2	2094	2094	196	23
PTGER3 (EP3)	**121**	**4**	**1**	**1**	**145**	**1**	**17**	**8**
WWC1	2094	5	2094	2094	2094	2094	2094	13
SLC2A4RG	13	6	2094	2094	2094	15	2094	11
FOXF1	107	7	71	467	1830	12	2094	88
NFKBIZ	32	8	2094	155	2094	2094	24	40
ZNF488	11	9	2094	2094	2094	2094	14	7
ZNF91	232	10	343	100	2094	2094	2094	64

C

nSCORE Rank								
Gene	ALL NT vs 6h	ALL NT vs 24h	LN428 NT vs 6h	LN428 NT vs 24h	LN827 NT vs 6h	LN827 NT vs 24h	U87 NT vs 6h	U87 NT vs 24h
PTGER3 (EP3)	**6562**	**22**	**31**	**5**	**1477**	**8**	**112**	**14**

Figure 2: EP3 is the master regulator of cellular resistance to TTFields. (A) GBM cell lines LN428, LN827, and U87 analyzed with heatmaps using NETZEN platform nSCORE at three time points: NT control group, day 7 of first cycle, and day 35 of the fifth cycle, when the cells have fully acquired resistance. Compared to NT control, day 7 and day 35 contained remarkably different global patterns in gene upregulation and downregulation, making it evident that gene signature changes began as early as day 7. **(B)** We used a regulatory network to rank a list of genes based on importance as a master regulator responsible for the cell's remodeling in response to TTFields through comparison of NT and day-35 time points. EP3 is shown to have consistently higher rankings across all cell lines, with its ranking rising as resistance develops from day 7 to day 35. **(C)** To determine whether resistant cells were selected or evolved to adapt to TTFields treatment and investigate EP3's role as master regulator, we observed a rapid increase in EP3 levels and ranking from 6 hours to 24 hours in LN428 and U87 cell lines. These findings suggest that resistance was developed through network remodeling and not selected.

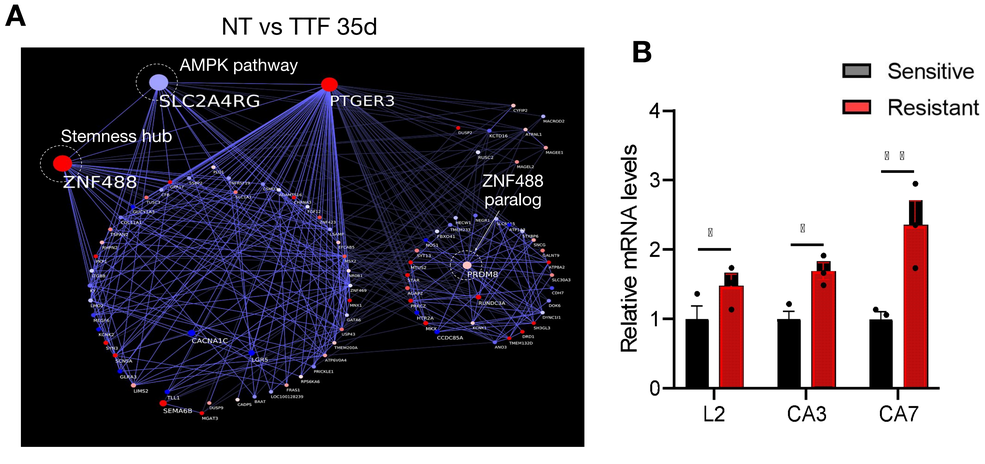


Figure 3: ZNF488 contributes to resistance and stemness in cells. (A) Used computational analysis to find EP3 and ZNF488, a stemness transcription factor, tightly connected to regulate resist TTFields treatment. **(B)** To back up our findings, we treated cells with TTFields and found that cell lines sensitive to TTFields have lower levels of ZNF488 mRNA and protein than resistant cell lines. ZNF488 is shown to play a role in GBM cell resistance to TTFields.

Will TMZ improve TTFields treatment or suppress it?

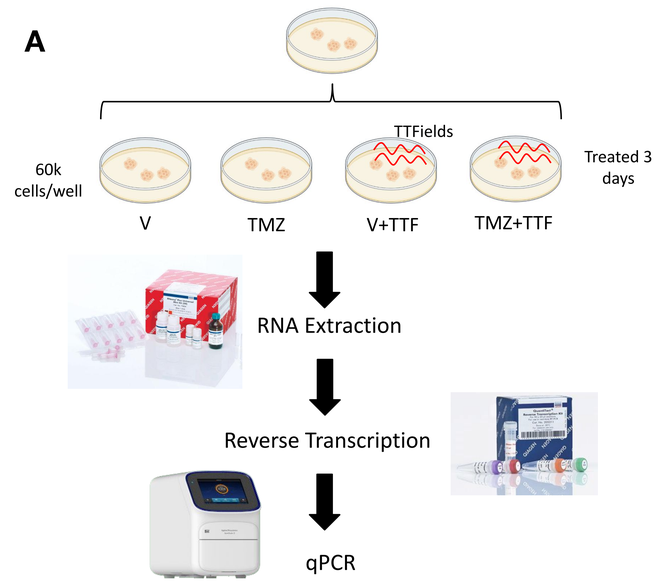
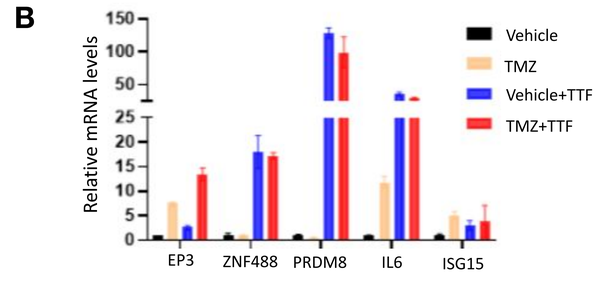


Figure 4: Combined treatment of TMZ and TTFields increases levels of EP3. (A) My workflow in executing this experiment to address the question regarding combined treatment efficacy. A cell plate split into 4 different plates undergoes RNA extraction, reverse transcription, and qPCR to achieve the data in Figure 4B. **(B)** After 3 days of TTFields treatment, we found mRNA levels of EP3 to increase with TMZ+TTF combination treatment. Levels of ZNF488 and PRDM8, the secondary regulators of resistance, stayed relatively the same. Combination treatment only increases EP3.



Conclusion

- EP3 is the top master regulator responsible for TTFields resistance
- EP3 recruits ZNF488 in the nucleus to counter TTFields treatment
- ZNF488, a transcription factor, contributes to cell resistance of TTFields
- EP3 and ZNF488 are potential targets for improving therapeutic efficacy of TTFields anti-cancer treatment
- The combination of TMZ+TTF increases levels of EP3, possibly with more resistance from cells; however, further experiments are needed to be done to confirm this finding

CONTACT US

bridge.usc.edu/bugs **David.Tran@med.usc.edu**