Nanosecond pulsed electric field treatment of tumor cell lines triggers Immunogenic Cell Death (ICD)

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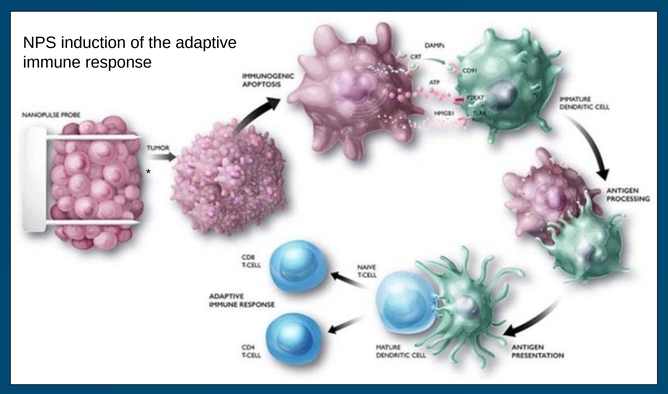
Abstract

Nano-Pulse Stimulation (NPS) is a non-thermal, localized application of ultrashort electric pulses in the nanosecond range that can trigger immunogenic cell death in treated tumors. We have demonstrated previously that the application of 400 pulses 100 ns long and 30 kV/cm in amplitude completely ablates treated orthotopic rat liver tumors within 2 weeks via apoptosis and initiates an immune response that inhibits secondary tumor growth in a CD8-dependent manner[1]. Here we show that NPS treatment results in the expression of three damage-associated molecular patterns (DAMPs) that play significant roles in immune signaling.

Methods

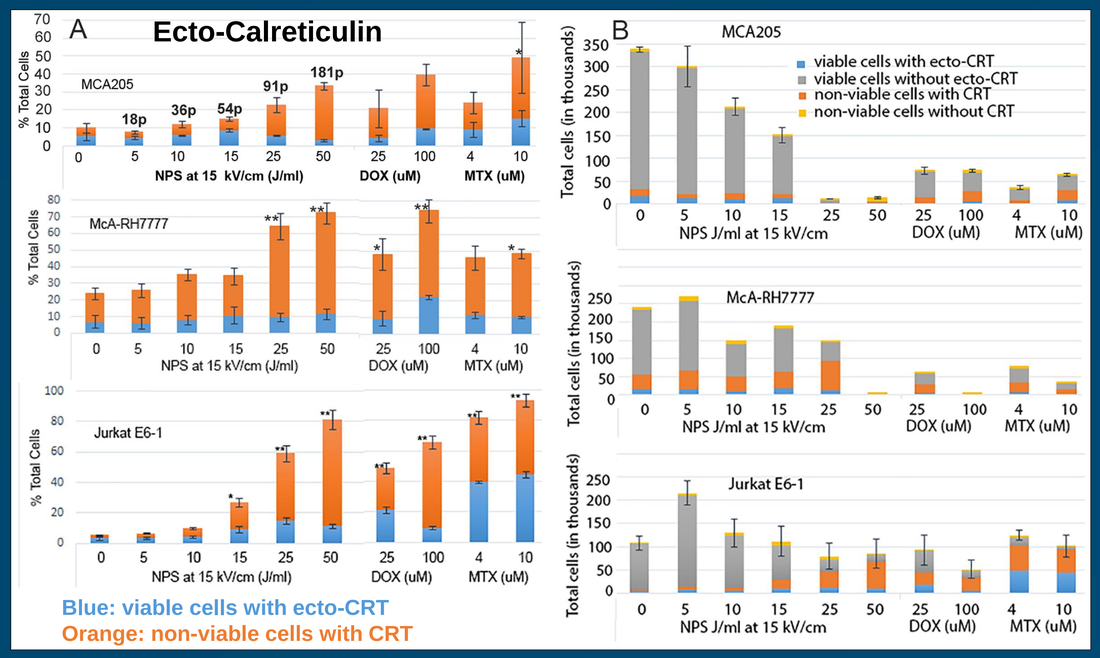
We treated three separate cancer cell lines (MCA205, McA-RH7777, Jurkat E6-1) with NPS. 1.25 million cells were suspended in 800 µl media and treated in a 4mm electroporation cuvette. Five total treatments were delivered ranging in energy from 5-50 J/mL. The pulse parameters were fixed (15 kV/cm, 100 ns, 2 pps) and energy delivery was controlled by varying the pulse number. 500,000 cells from each treatment group and untreated cells were seeded into a 24-well plate and incubated at 37° C for 24-hours. Cell culture supernatants were collected to measure levels of HMGB1 and ATP. Cells were also harvested and the expression levels of cell surface calreticulin were determined using flow cytometry.

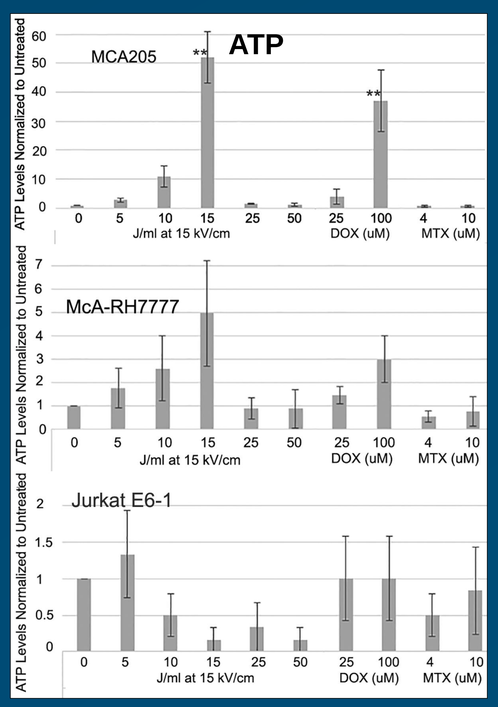


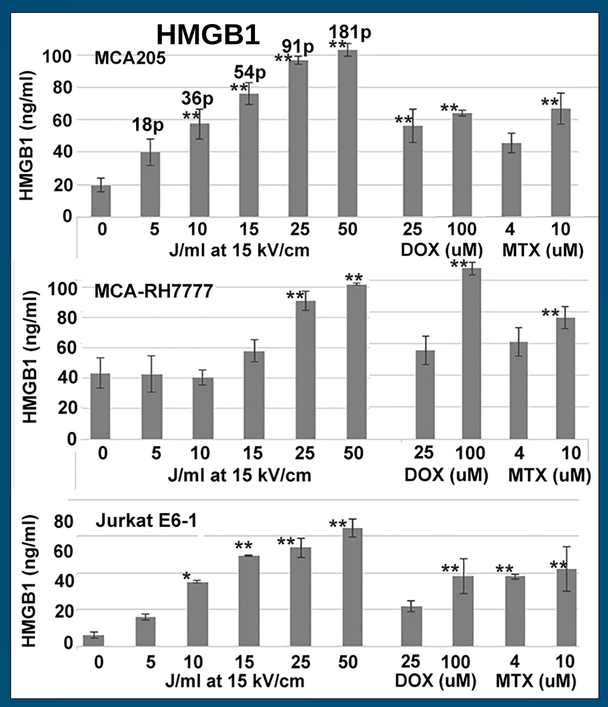
References

[1] Nuccitelli, R., et al. (2015) Nanoelectroablation of Murine Tumors Triggers a CD8-Dependent Inhibition of Secondary Tumor Growth. PLos One 10(7):e0134364

Results







The initiation of apoptosis in cultured cells is greatest at 15 kV/cm and requires 50 A/cm^2. Reducing this current inhibits apoptosis. We measured the three DAMPs 24-hours after treatment. The expression of cell surface CRT increased in an energy-dependent manner in the NPS treated samples. Expression levels reached or exceeded the expression levels in the majority of the anthracycline treated samples at energies between 25-50 J/mL. Secreted ATP peaked at 15 J/mL and then rapidly declined at 25 J/mL. HMGB1 release increased as treatment energy increased and at energies between 10-25 J/mL reached levels comparable to the anthracycline-treated groups

Conclusion

Nano-Pulse Stimulation triggers the expression of 3 key DAMPs at levels comparable to Doxorubicin and Mitoxantrone, two known inducers of immunogenic cell death (ICD). Therefore, we conclude that NPS is a bona fide physical inducer of immunogenic cell death. Treatment energies ranging from 10-20 J/mL induce ICD *in vitro.* Further studies will be needed to determine the optimal pulse parameters and energy for triggering apoptosis in solid tumors.