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Nanosecond pulses electric field (nsPEF) treatment for peritoneal metastases from colorectal cancer

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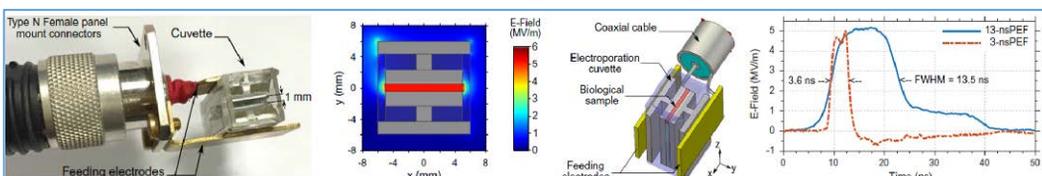
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INTRODUCTION :

- The majority of patients with peritoneal metastases (PM) from colorectal cancer (CRC) is considered unresectable at the time of diagnosis .
- High-intensity pulsed electric fields with nanosecond durations (3-300ns; nsPEFs)** have emerged as a promising tool for tumor ablation. The specific type of **cell death** that occurs following the application of nsPEFs is under investigation by several groups. They have shown effects on cell signalling through calcium and cell death pathways, mitochondria and cytoskeleton.
- This aim of this study was to analyse the effects of nsPEFs on CT-26 cells.**

MATERIELS / METHODS :

- Cancer cell lines : colon carcinoma cell line / **Luciferase-expressing murine CT-26 cells** .
- Animal : 4 weeks old Balb-c mice (C. River Lab., France) were grafted with 40 μ l of 30 000 CT-26 cells by intraperitoneal injection. Luciferase expression was monitored by bioluminescence imaging for 15 days.



- nsPEF exposure system : The generator (FPG 20, FID Technology, Germany) delivered 10ns pulses with 50kV/cm amplitude. The biological cuvette (plastic chamber containing two opposing flat aluminium electrodes spaced with a 1 mm gap, electrode dimensions : 10 mm large and 10 mm high) was connected to the coaxial cable using a custom-made adapter with two plated electrodes soldered on an N-connector.

MATERIELS / METHODS :

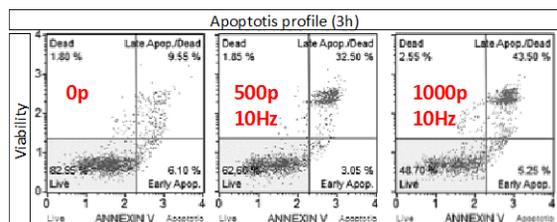
Survival and apoptosis analysis of CT-26

Immediately after exposure, the cells (n=3 for each parameter) were transferred to Eppendorf tubes and volume was completed to 100 μ l with complete medium. They were loaded with 100 μ l of Muse™ Annexin V & Dead Cell reagent (MCH100105, Millipore) for 20 min at room temperature. Determination of the apoptosis index (by Annexin V) and cell viability (by 7-AAD) was simultaneously measured by fluorescence using a flow cytometer (Muse® Cell Analyzer, Millipore) at 45 min and 3 hours post-exposure.

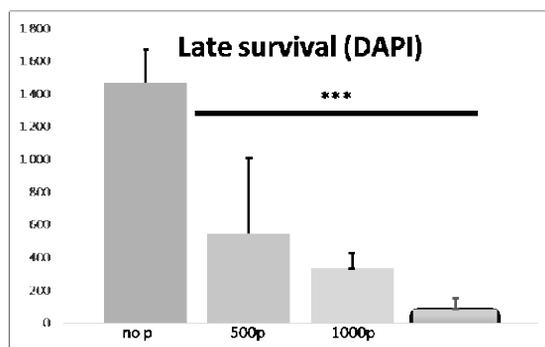
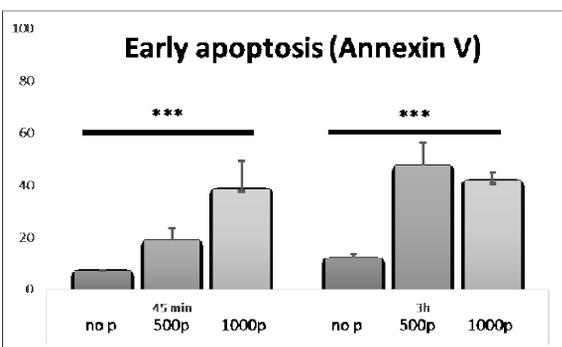
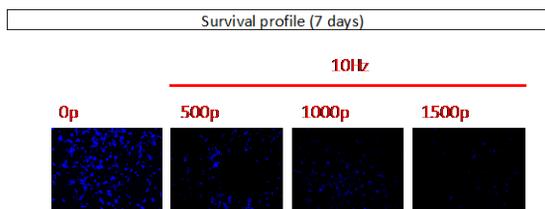
250 cells/well were reseeded after nsPEF exposure on a glass coverslip in complete medium for 3 to 7 days. They were fixed 20 min with 4% paraformaldehyde and permeabilized 15 min with 0.1% Triton in PBS. After labelling with 20 μ m/ml DAPI (Sigma, D9542), fluorescent nuclei were counted in random fields with Fiji/ImageJ (NIH).

RESULTATS

A



B



In vitro exposition of CT-26 cells to 10ns pulses induces cell death by apoptosis (A) and poor survival after 7 days post exposure (B). Three microscopic fields were arbitrarily chosen and the average DAPI+ cell number was determined (*** $p < 0.01$). Experimental data are expressed as mean values \pm SEM

CONCLUSION and PERSPECTIVES

- We have demonstrated that these CT-26 cells are sensitive *in vitro* to 10ns nsPEF.
- *In vivo* electrodes are now designed by our group in order to facilitate the exposure in mouse model.
- nsPEF exposure can be studied in combination with classical therapy to evaluate the anti-tumoral synergic effects and the immune response.

