

## **Programmed cell death induced by modulated electro-hyperthermia**

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# Programmed cell death induced by modulated electro-hyperthermia

## Abstract

**Background:** Modulated electro-hyperthermia (mEHT) is a non-invasive technique for targeted tumor treatment.

**Method:** HT29 human colorectal carcinoma cell line xenografted to both femoral regions of BalbC/nu/nu mice treated with a single shot OTM treatment. Histomorphologic, and immunohistochemical analysis TUNEL assay and R&D Apoptosis array were performed on tissue samples.

**Results:** mEHT caused a selective tumor demolition. An up regulation of TRAIL-R2 and FAS was observed. Cleaved caspase-3 positive cells appear at the tumor periphery. Cytochrome c and AIF release was observed in line with massive TUNEL positivity.

**Conclusion:** In HT29 colorectal cancer xenograft mEHT caused massive caspase independent cell death.

## Background

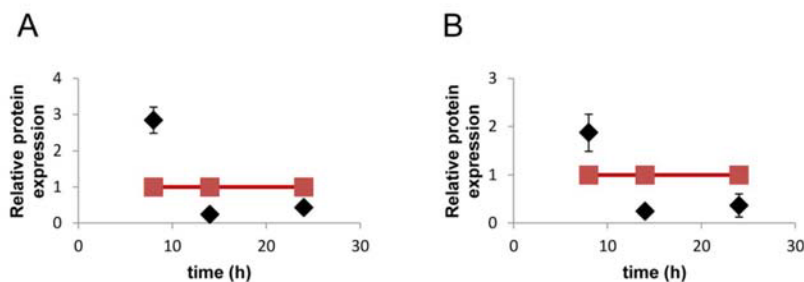
Modulated electro-hyperthermia (mEHT) is a non-invasive technique for targeted tumor treatment [1-4]. The capacitive coupled modulated radiofrequency enriches in the tumor tissue, because of its dielectric differences [5, 6], without harming the surrounding non-malignant tissues. The possible mechanism of action of conventional hyperthermia on tumor models was previously slightly investigated and have not been fully evaluated [7]. Already it was shown that mEHT has non-temperature dependent effect beside the temperature dependent one [8]. Here our aim was to detect the possible role of mEHT in tumor cell death.

## Method

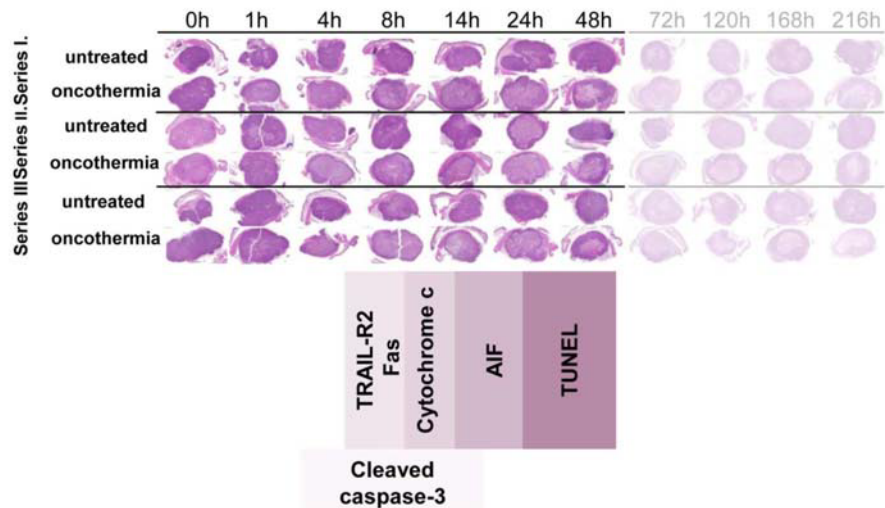
HT29 human colorectal carcinoma cell line xenografted to both femoral regions of BalbC/nu/nu mice were treated with a single shot OTM treatment (LabeHY, Oncotherm Ltd, Páty, Hungary) for 30 minutes into the approx. 1.5 cm diameter tumors. Sampling was made after 0, 1, 4, 8, 14, 24, 48, 72, 120, 168, 216 h in 3 mice in each group by keeping 5 untreated animals. The temperature measurement was carried out during the treatment using optical probes (Luxtron FOT Lab Kit, LumaSense Technologies, Inc. CA, USA). The treated tumor core (41-42°C was during the treatment) the surface subcutaneously, the untreated tumor core and the rectal temperature were measured. Histomorphologic (H&E), immunohistochemical analysis by cleaved caspase-3 (Cell Signaling, Danvers, MA), TRAIL-R2 (Cell Signaling), cytochrome c (Cell Signaling), AIF (Cell Signaling) was completed on formalin fixed paraffin embedded tissue microarrays (TMA, TMA Master, 3DHISTECH Ltd., Budapest, Hungary) prepared from all samples. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Invitrogen, Carlsbad, CA) was performed on TMA and 24h and 48h post-treatment on the whole sections. R&D Apoptosis array (R&D, Minneapolis, MN) was carried out on the 8h, 14h and 24h treated and 24h untreated samples. Results were analyzed using digital microscopy and were evaluated by ImageJ.

## Results

Modulated EHT caused a selective tumor demolition proceeding from the tumor centre. An up regulation of TRAIL-R2 and FAS was observed 8 h post treatment.



**Figure 1.** Relative protein expression of TRAIL-R2 (A) and Fas (B). An elevated expression can be noticed 8h post-treatment in both TRAIL-R2 and Fas proteins. The black rectangles show the treated sample relative protein expression while the red represents the relative control



**Figure 2.** The summary of the possible mechanisms of actions of programmed cell death can be seen. Based on the immunohistochemistry results 8h post-treatment elevated TRAIL-R2 expression was observed, 8-14h post-treatment mitochondrial cytochrome c release was detected, in line with this AIF nuclear translocation was revealed on the 14-24h samples. Between 24-48h massive DNA fragmentation was identified by TUNEL assay

Cleaved caspase-3 positive cells (mostly leucocytes) appeared only at the tumor periphery 4-14h. Cytochrome c release was observed 8-14 h post treatment. AIF nuclear translocalisation occurred 14-24h. Massive TUNEL positivity developed 24-48h post treatment. Heavy myeloperoxidase and CD3 positive leukocyte infiltration ring showed 72-216 h possibly correlates to the tumor elimination.

## Results

In HT29 colorectal cancer xenograft mEHT caused massive cell death, the occurrence of a caspase independent, AIF dependent programmed cell death subroutine.

## Conflict of interest

Authors declare no conflict of interest in this project.

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