


**P-04: Meggyeshazi Nora, Andocs Gabor, Krenacs Tibor (2012) Programed cell death induced by modulated electro-hyperthermia**

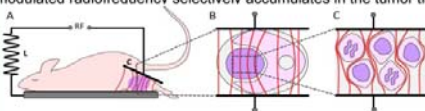


## Programmed cell death induced by modulated electro-hyperthermia


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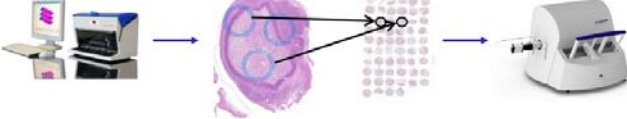
**Background:** Modulated electro-hyperthermia (mEHT) is a non-invasive technique for targeted tumor treatment. The mEHT generated capacitive coupled modulated radiofrequency selectively accumulates in the tumor tissue without major effect in the surrounding normal tissues.



**Figure 1:** Scheme of the experimental set up and hypothetical effect of modulated electro-hyperthermia treatment. The radiofrequency (RF) generated electric field (red lines) is enriched in the tumor implant which is part of the circuit (A). Cross section through the mid femoral region filled up by the tumor (solid lump) accumulating the current (B), which can not pass through cell membranes as it is depicted at the microscopic level (C). However, the electric field alternating by 13.56 MHz generates heat and interacts with ions in the extracellular space and any dipole molecular groups (non-thermal effect) causing them to rotate both in the matrix and cell membrane receptors. As a result of enhanced electric field and deregulated adaptive pathways in tumors, mEHT treatment can significantly interfere with the fate of tumor cells than normal cells and may trigger tumor cell destruction.



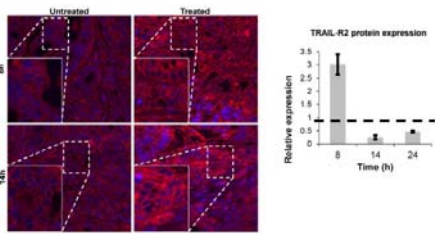
**Figure 2:** Double tumor model. Temperature (sub-cutaneous, tumor core, control side and rectal) was controlled <math>\pm 0.2^{\circ}\text{C}</math>.



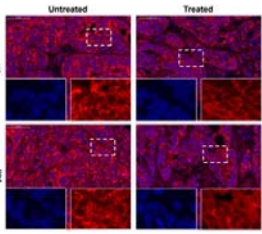
**Figure 3:** We have prepared tissue microarray (TMA), standard array selection based on digital slides. Followed by several immunofluorescence and immunohistochemistry with TRAILR2, AIF, Cytochrome-C, and cleaved caspase-3 (all from Cell Signaling) and performed TUNEL assay. The slides were digitized.

**Method:** HT29 human colorectal carcinoma cell line xenografted to both femoral region of BalbC/nu/nu mice was treated when reaching ~1.5 cm by using a single shot mEHT treatment (LabEHY, Oncotherm Ltd, Páty, Hungary) for 30 minutes. Sampling was made after 0, 1, 4, 8, 14, 24, 48, 72, 120, 168, 216 h in 3 mice each group by keeping 5 untreated animals. Histomorphologic, immunohistochemical and TUNEL assay results were tested in digital slides and analyzed semi-quantitatively. An apoptosis protein array was used to screen 35 apoptosis related proteins, results were evaluated using the ImageJ software.

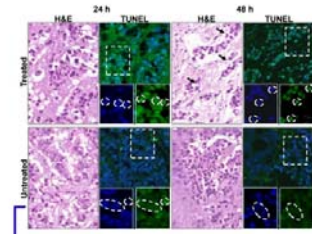
**Results:** mEHT caused programmed cell death related destruction from the tumor centre. TRAILR2 was up-regulated 8h post treatment. Cleaved caspase-3 positive cells appeared only at the tumor periphery between 4-14h. AIF nuclear translocation at 14h and cytochrome c release from the mitochondria at 8-14h and massive TUNEL positivity at 24-48h indicated DNA fragmentation.



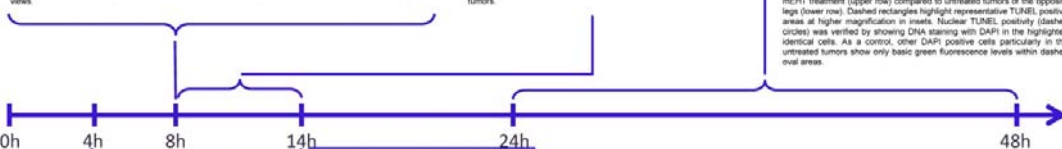
**Figure 4:** TRAILR2 expression in the tumor cell membranes is elevated both 8 and 14 hours post-treatment compared to the control side tumors. The elevation at 14h post-treatment appears only at the tumor periphery but not in the center, while evenly low TRAILR2 levels are seen throughout the control tumor. Insets show high power views.

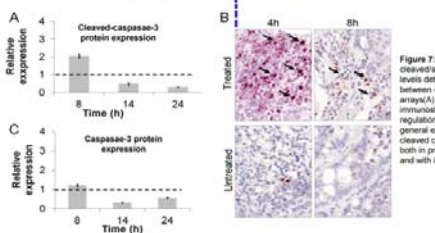


**Figure 5:** Cytochrome c was released into the cytoplasm from 8h post-treatment with a peak at 14h which disappeared by 24h on the treated sides. It remained mitochondrial in the untreated tumors.

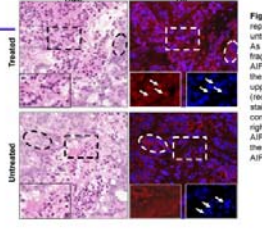


**Figure 6:** Both nuclear shrinkage and apoptotic bodies (arrows; H&E staining) as well as DNA fragmentation proved by the TUNEL assay (green fluorescence) became significantly elevated both at 24h and 48h after mEHT treatment (upper row) compared to untreated tumors of the opposite legs (lower row). Dashed rectangles highlight representative TUNEL positive areas at higher magnification in insets. Nuclear TUNEL positivity (dashed circles) was verified by showing DNA staining with DAPI in the highlighted identical cells. As a control, other DAPI positive cells particularly in the untreated tumors show only basic green fluorescence levels within dashed oval areas.





**Figure 7:** Elevated cleaved/activated-caspase-3 levels detected as early as between 4-8h both in protein arrays (A) and after immunostaining (B) and down-regulation soon afterwards. Low general expression of non-cleaved caspase-3 was found both in protein arrays (C) and with immunohistochemistry.



**Figure 8:** H&E morphology of representative areas of treated and untreated tumors 14h post-treatment. As an early sign of later DNA fragmentation nuclear translocation of AIF (red) is clearly seen at this stage in the treated side only (dotted squares, upper right). This colocalization of AIF (red) and nuclear DNA (blue, DAPI) staining is missing in the untreated control tumor (dotted squares, lower right), while the normal mitochondrial AIF is still present. Dotted circles show the normal mitochondrial localization of AIF.

**Conclusion:** In HT29 colorectal cancer xenograft mEHT (modulated electro-hyperthermia) caused programmed cell death. DNA fragmentation followed rather a caspase independent and AIF dependent subroutine with cytochrome c release.

**Acknowledgement:** Iveti Teleki, Edit Parsch, Péter Balla, Gergő Kiszner

**In memoriam Réka Szász**