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Early changes in protein expression releated to modulated electro-hyperthermia

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Early changes in protein expression releated to modulated electro-hyperthermia

Background

Modulated electro-hyperthermia (mEHT) is a widely used 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]) without harming the sorrunding non-malignant tissues. Beside the temperature dependent effect mEHT causes in the tumor tissue, it has a non-temperature dependent tumor destruction effect, which is three times higher than the conventional hyperthermia with the temperature dependent outcome only [6]. Here our aim was to study early changes in protein expression either related or not to the temperature changes in tumors with a single shot of mEHT.

Method

HT29 human colorectal carcinoma cell line xenografted to both femoral region of BalbC/nu/nu mice. Tumors (approx.. 1.5 cm diameter) were treated with a single shot mEHT treatment (LabEHY, Oncotherm Ltd., Páty, Hungary) for 30 minutes. Temperature measurement was carried out during the treatment in the treated tumor core and subcutaneously, in the opposite (treated control) tumor core and rectally. The treated tumor core temperature was between
41-42 ^{o}C during the treatment. Sample was taken 0, 1, 4, 8, 14, 24 and 48 h after the treatment, each group containing 3 mice alongside with 2 untreated control animals (sample was taken simultaneously with the 24h treated group). Human genome U133 Plus 2.0 Array (Affymetrix Inc., Santa Clara, CA) was used on the 4h treated animals' both samples (teated and untreated side) and on the 24h untreated control samples to identify treatment related mRNA alterations. The results were analyzed by Bioconductor software. R&D Apoptosis array (R&D, Minneapolis, MN) was performed on the 8, 14 and 24 h treated and the 24h untreated control tissue samples. 35 apoptosis related proteins were observed. Results were analyzed by ImageJ. Immunohistochemistry was carried out on formalin fixed paraffin embedded (FFPE) tissue microarray (TMA) (3D HISTECH Ltd., Budapest, Hungary) slides to confirm and to identify the localization of the previously identified proteins. On whole cross sections Terminal deoxynucleotidyl transferase dUTP nick end labling (TUNEL) assay (Invitrogen, Carlsbad, CA) was carried out 24 and 48 h after mEHT treatment. The slides were digitalized with Panoramic Scanner and analyzed with Panoramic Viewer software (both form 3D HISTECH Ltd., Budapest, Hungary).

Results

According to the mRNA chip array, there were 48 genes showing significant differential expression related to the treatment, including heat shock protein isotypues (hsp70, hsp90, hsp60 and hsp40).

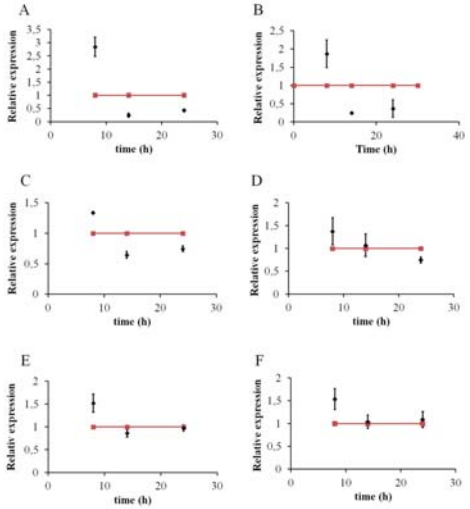

Figure 1. Relative protein expression of TRAIL-R2 (A), Fas (B), FADD (C), Bax (D), SMAC/Diablo €, HTRA2/Omi (F). The black rectangles show the treated sample relative protein expression while the red represent the relative control

Using apoptosis protein expression arrays the up regulation of death receptors (TRAILR2, Fas) and FADD (Fas associated death domain), Bc12 super family proteins (Bax.), mitochondrial apoptosis regulatory proteins (SMAC/Diablo, HTRA2/Omi) were observed 8h post treatment. In correlation with mRNA levels of heat shock proteins hsp70 and hsp60 were detected too (the array did not include hsp40 or hsp90).

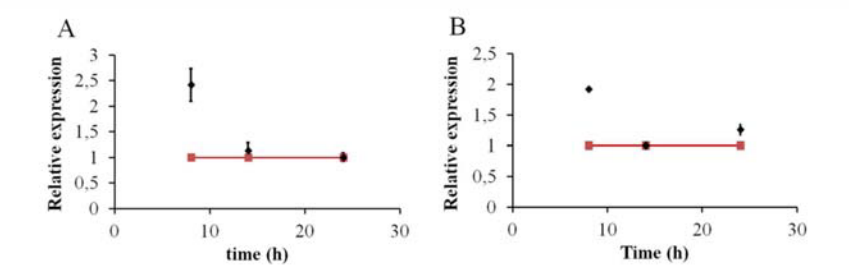

Figure 2. Relative protein expression of hsp60 (A), hsp70 (B). The black rectangles show protein levels in the treated samples, while the red represent the relative control levels

Elevation of hsp70 protein was also shown with immunohistochemistry starting from 14h post treatment. In situ protein detection using immunohistochemistry also confirmed the up regulation of the death receptor TRAIL-R2 between 8-14h post treatment along with cytochrome C release from the mitochondria to the cytoplasm between 8-10 h the nuclear translocation of apoptosis inducing factor (AIF) 14h post treatment. In line with these findings, TUNEL assay proved significant DNA fragmentation and elevated numbers of apoptotic bodies 24-48h post treatment.

Conclusion

A single shot mEHT treatment resulted in the up-regulation of a range of proteins related to apoptosis induction and heat shock response in HT29 colorectal cancer xenograft within 24 hours post treatment.

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