Conference Paper

Early Changes in mRNA and Protein Expression Related to Cancer Treatment by Modulated Electrohyperthermia

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Modulated electrohyperthermia (mEHT), generated by capacitive coupled, modulated 13.56 MHz radiofrequency, is a noninvasive technique for targeted tumor treatment based on elevated ion concentration and electric admittance in malignant tumors. In this study, we tested early changes in protein expression related to tumor destruction upon a single shot of 30-minute mEHT treatment of xenografted human colorectal cell line (HT29) implanted into the femoral region of Balb/c nu/nu mice. Treatment-related mRNA expression profiling was done using the human genome U133 Plus 2.0 Arrays. Apoptosis protein arrays and immunohistochemistry were performed for validating changes at the protein level. The mEHT treatment resulted in major expression changes in 48 genes including several heat-shock proteins. Apoptosis protein arrays revealed the upregulation of death receptors, Bcl-2 superfamily mitochondrial apoptosis regulatory proteins, and heat-shock proteins, which were also confirmed *in situ*. Within 24-hour post-treatment, mEHT resulted in the upregulation apoptosis induction and heat-shock-related gene and protein expression in HT29 colorectal cancer xenografts contributing to tumor destruction.

1. Background

Modulated electrohyperthermia (mEHT) is a widely used noninvasive 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 surrounding nonmalignant tissues. Beside the temperature-dependent effect mEHT causes in the tumor tissue, it has a nontemperature-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 treated with a single shot of mEHT.

2. Method

HT29 human colorectal carcinoma cell line is xenografted to both femoral regions of Balb/c 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°C during the treatment. Sample was taken at 0, 1, 4, 8, 14, 24, and 48 h after the treatment with each group containing 3 mice alongside with 2 untreated control animals (sample was taken simultaneously with the 24 h treated group).

FIGURE 1: Relative protein expression of TRAIL-R2 (a), Fas (b), FADD (c), Bax (d), SMAC/Diablo (e), and HTRA2/Omi (f). The black rectangles show the treated sample relative protein expression while the red represent the relative control.

The Governmental Ethical Committee approved the study under no. 22.1/609/001/2010. Human genome U133 Plus 2.0 Array (Affymetrix Inc., Santa Clara, CA) was used on the 4h treated animals' both samples (treated and untreated sides) and on the 24h untreated control samples to identify treatment-related mRNA alterations. The results were analyzed by Bioconductor software. R ampersand D Apoptosis array (R ampersand D, Minneapolis, MN) was performed on the 8, 14, and 24 h treated and the 24 h untreated control tissue samples. Thirty five 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 labeling (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 from 3D HISTECH Ltd., Budapest, Hungary).

3. Results

According to the mRNA chip array, there were 48 genes showing significant differential expression related to the

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.

treatment, including heat-shock protein isotypes (hsp70, hsp90, hsp60, and hsp40).

Using apoptosis protein expression arrays, the up-regulation of death receptors (TRAIL-R2, Fas) and FADD (Fas-associated death domain), Bcl2 super-family proteins (Bax), mitochondrial apoptosis regulatory proteins (SMAC/Diablo, HTRA2/Omi) were observed 8 h post treatment (Figure 1). 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).

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

4. Conclusion

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

Conflict of Interests

Authors declare no conflict of interests in this paper.

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