Conference Paper

Programmed Cell Death Induced by Modulated Electrohyperthermia

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1. Background

Modulated electrohyperthermia (mEHT) is a noninvasive 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 nonmalignant tissues. The possible mechanism of action of conventional hyperthermia on tumor models was previously slightly investigated and has not been fully evaluated [7]. Already it was shown that mEHT has nontemperature dependent effect beside the temperature dependent one [8]. Here, our aim was to detect the possible role of mEHT in tumor cell death.

2. Method

HT29 human colorectal carcinoma cell line xenografted to both femoral regions of BalbC/nu/nu mice was treated with a single shot OTM treatment (LabEHY, Oncotherm Ltd, Páty, Hungary) for 30 minutes of approximately 1.5 cm diameter tumors. Sampling was made after 0, 1, 4, 8, 14, 24, 48, 72, 120, 168, and 216 h in 3 mice, 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 temperature was 41–42°C during the treatment. Histomorphologic (H&E), immunohistochemical analysis by cleaved caspase-3 (Cell Signaling, Danvers, MA), TRAIL-R2 (Cell Signaling), cytochrome c (Cell Signaling), and AIF (Cell Signaling) were 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 at 24 h and 48 h after treatment of whole sections. R&D Apoptosis array (R&D, Minneapolis, MN) was carried out on the 8 h, 14 h, and 24 h treated and 24 h untreated samples. Results were analyzed using digital microscopy and were evaluated by ImageJ.
3. Results

Modulated EHT caused a selective tumor demolition proceeding from the tumor centre. An upregulation of TRAIL-R2 and FAS was observed 8 h after treatment (Figure 1).

Cleaved caspase-3 positive cells (mostly leucocytes) only appeared at the tumor periphery at 4–14 h. Cytochrome c release was observed at 8–14 h after treatment. AIF nuclear translocalisation occurred at 14–24 h (Figure 2). Massive TUNEL positivity develops at 24–48 h after treatment. Heavy myeloperoxide and CD3 positive leukocyte infiltration ring was observed between 72–216 h, which possibly correlates to the tumor elimination.

4. Conclusion

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

Conflict of Interests

The authors declare no conflict of interests in this project.
References


