Hindawi Publishing Corporation
Conference Papers in Medicine
Volume 2013, Article ID 187835, 3 pages
http://dx.doi.org/10.1155/2013/187835

Conference Paper

Programmed Cell Death Induced by Modulated Electrohyperthermia

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Received 22 January 2013; Accepted 7 July 2013

Academic Editors: G. Baronzio, M. Jackson, and A. Szasz

This Conference Paper is based on a presentation given by Meggyesházi Nóra at "Conference of the International Clinical Hyperthermia Society 2012" held from 12 October 2012 to 14 October 2012 in Budapest, Hungary.

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Background. Modulated electrohyperthermia (mEHT) is a noninvasive technique for targeted tumor treatment. *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. Histomorphologic, immunohistochemical analysis TUNEL assay, and R&D Apoptosis array were performed on tissue samples. *Results*. mEHT caused a selective tumor demolition. An upregulation 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.

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 the treated tumor surface subcutaneously, the untreated tumor core and the rectal temperature was measured. The treated tumore 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.

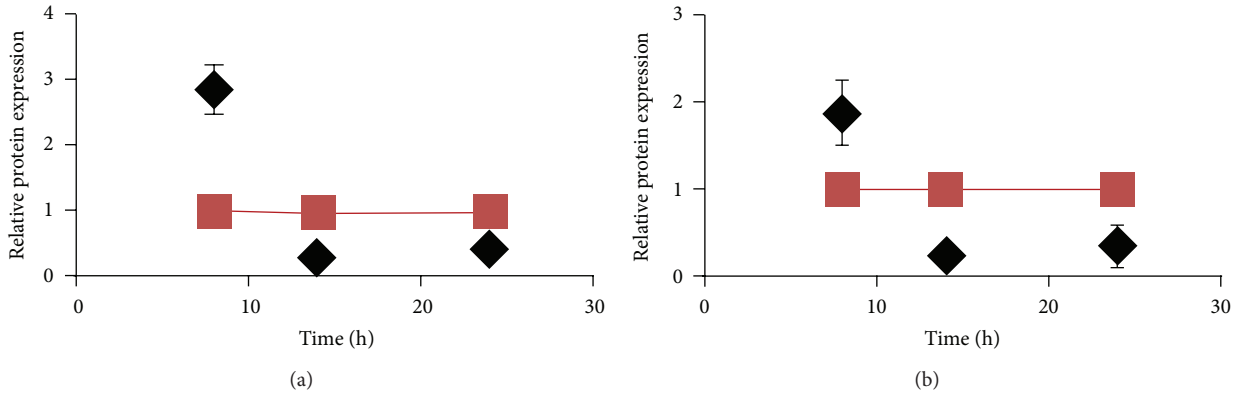

Figure 1: Relative protein expression of TRAIL-R2 (a) and Fas (b). An elevated expression can be noticed 8 h after treatment in both TRAIL-R2 and Fas proteins. The black rectangles show the treated sample relative protein expression, while the red ones represent the relative control.

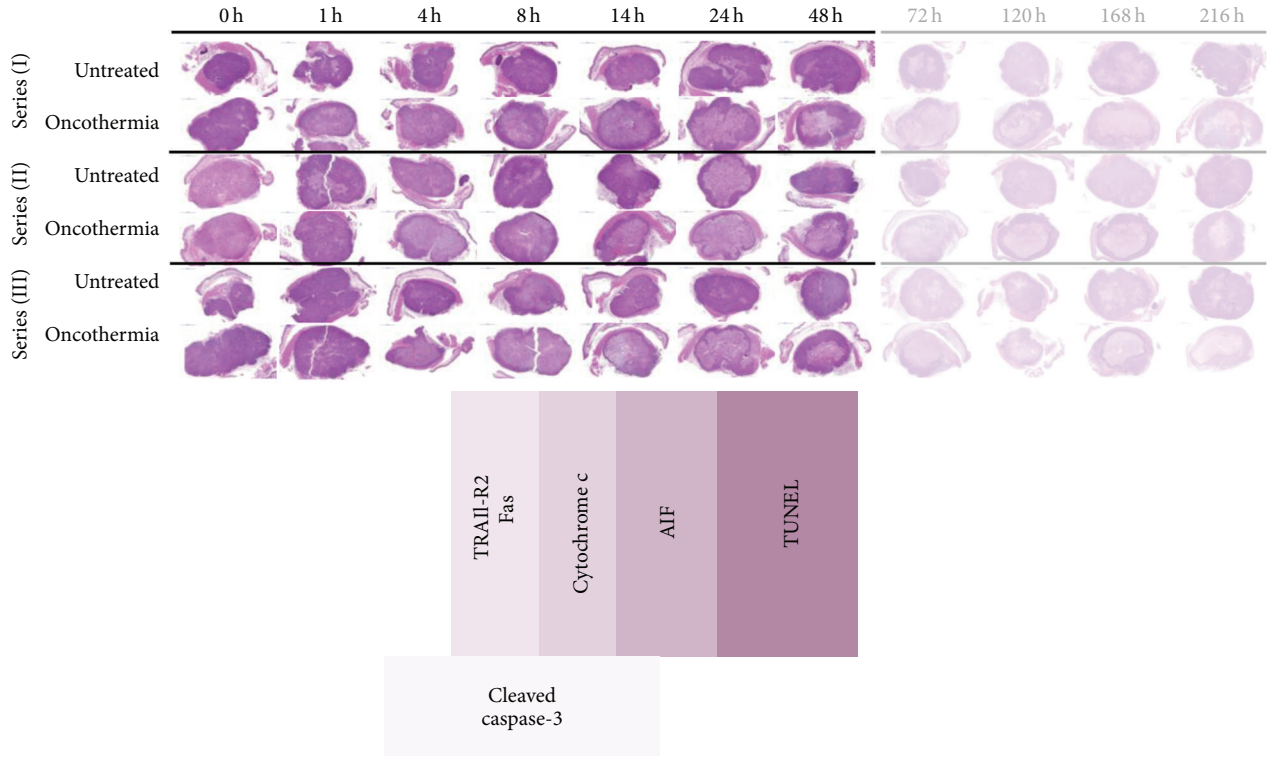

Figure 2: The summary of possible mechanism of action of programmed cell death can be seen. Based on the immunohistochemistry results, 8 h posttreatment elevated TRAIL-R2 expression was observed, 8–14 h posttreatment mitochondrial cytochrome c release was detected, and in line with this AIF nuclear translocation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

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 between72–216 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.

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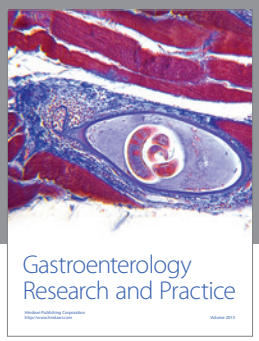

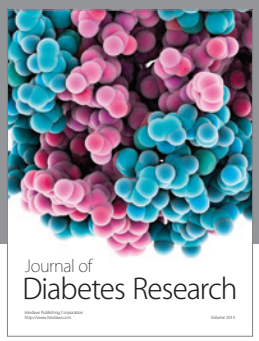

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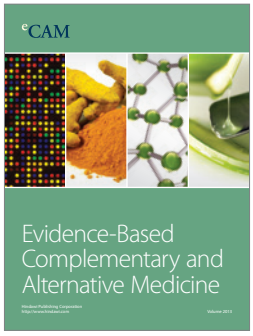

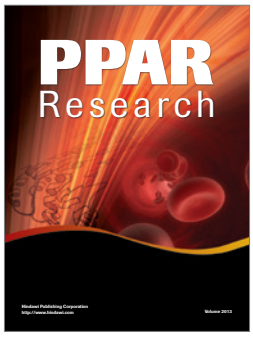

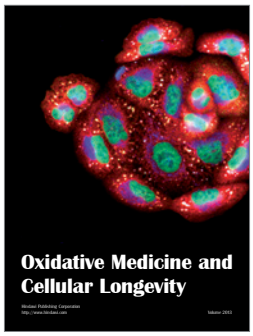

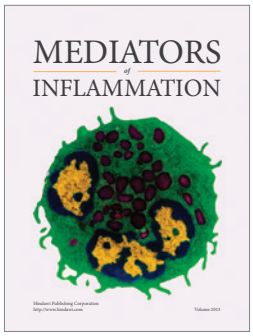

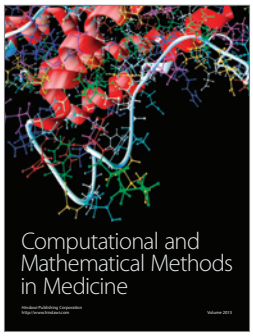

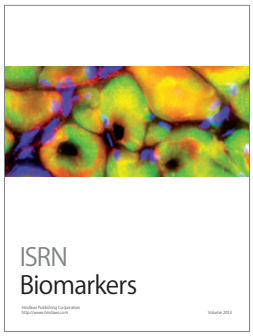

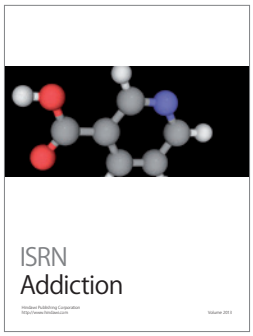

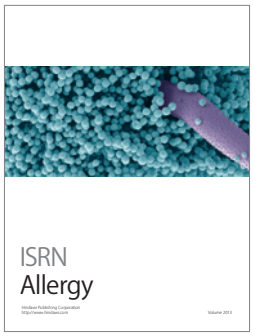

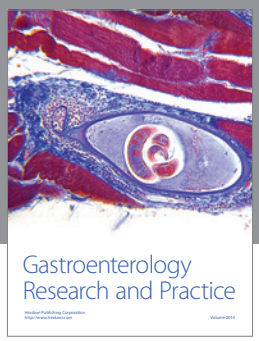

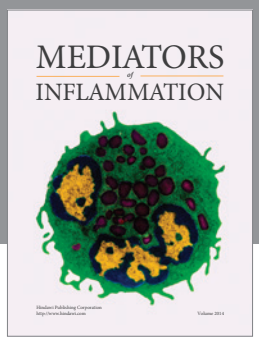

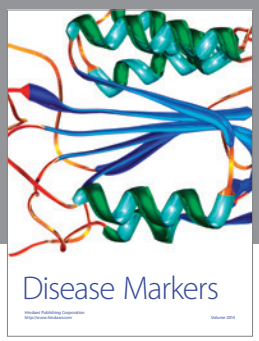

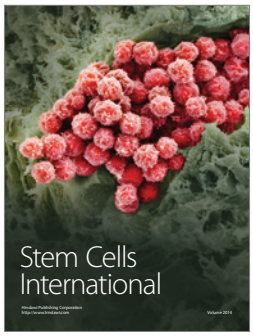

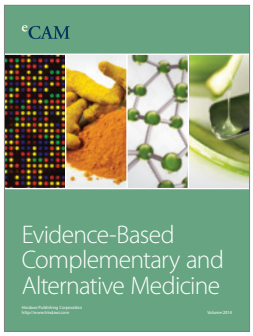

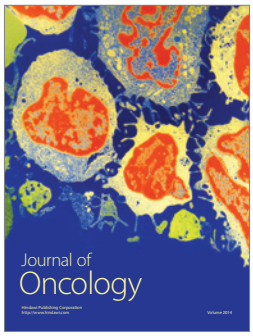

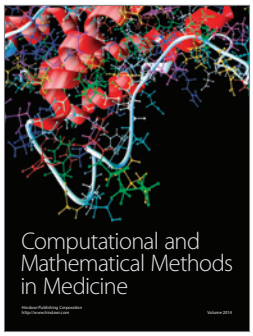

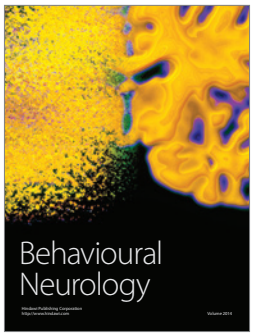

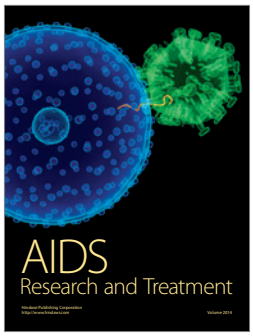

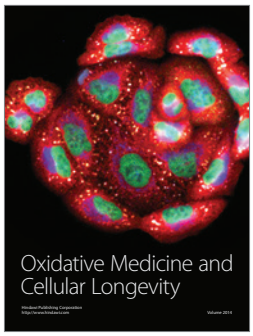

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