

Bystander effect of oncothermia

Andocs G¹, Meggyeshazi N², Okamoto Y¹, Balogh L³, Szasz A⁴

(4) Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, Tottori, 4-101 Minami, Koyama-cho., tottori Pref., Japan

(5) 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, 1085-Budapest, Ulloi ut 26., Hungary

(6) “Frederic Joliot Curie” National Research Institute for Radiobiology and Radiohygiene 1221-Budapest, Anna u. 5., Hungary

(7) Department of Biotechnics, Szent Istvan University, 2100-Godollo, Pater K. u. 1., Hungary
Corresponding author: Gabor Andocs gandocs@gmail.com

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Background

Oncothermia (OTM) is an electro-hyperthermia modality, a long time (since 1989) applied method, [1], used successfully in human oncology [2]. OTM changes the paradigm of hyperthermia by targeted microscopic heat-liberation at the membrane of the malignant cells. This method creates inhomogeneous heating, and the microscopic temperature greatly differs far from the thermal equilibrium. The tumor destruction efficacy and the role of temperature independent effects of the OTM were proven earlier by a laboratory research, and were presented elsewhere [3], [4]. Bystander effect (abscopal effect) means that a local tumor treatment can affect the behavior of the far distant metastases. It was first discovered by radio-oncologists and remained a highly controversial topic until recent years. [5], [6]. Intensive research is being conducted to reveal the immunobiological basis [7], [8], [9] and the mechanism of the action of this effect [10] and to use the benefits in the regular oncological practice. Our objective is to show the newest results of oncothermia in research of the bystander effect.

Materials and methods

Animal model

HT29 human colorectal carcinoma cell line derived xenograft tumor model in nude mice. The use of the mice and the procedures used in this study were approved by the Animal Experiment Ethical Committee of National Research Institute for Radiobiology and Radiohygiene.

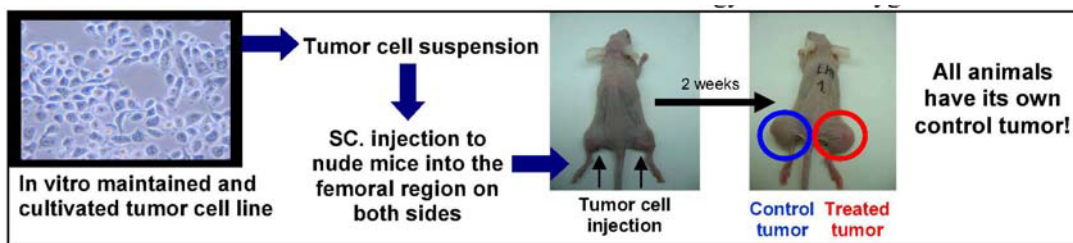


Figure 1. Process of the tumor induction of the experimental animals

Experimental setup and treatment

A single shot 30 min oncothermia treatment was done, reaching maximum 41-42°C intratumoral temperature, using the LabEHY system (Oncotherm Ltd. Germany), under precise tumor temperature control using fluoroptic temperature measurement system (Lumasense, Luxtron m3300).



Figure 2. Experimental setup of the oncothermia treatments in the laboratory

Study design: Time course study was performed. After a single shot treatment, sampling was made after 0, 1, 4, 8, 14, 24, 48, 72, 120, 168, 216 hours. 3 mice were sacrificed at each time point, keeping 5 sham treated animals.



Figure 3. All the oncothermia-treated experimental animals involved in this study

Tumor sample processing: At the time of the sampling the single-treatment animals were sacrificed and both the control and treated tumors were removed and studied in pairs.

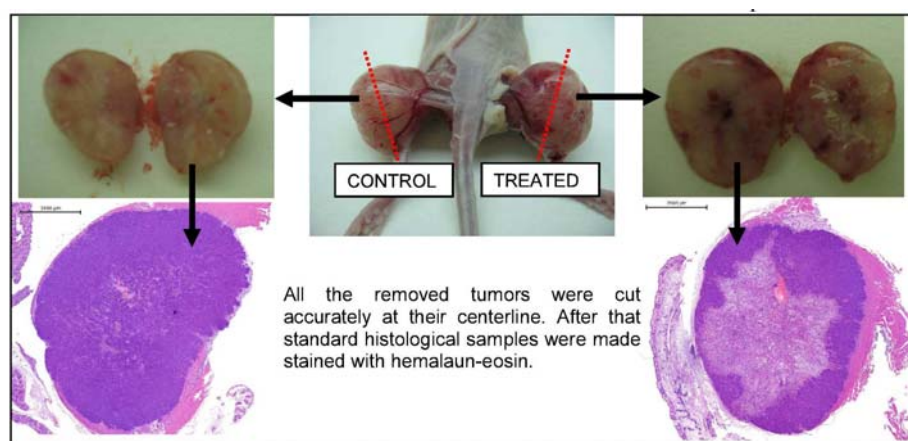


Figure 4. Method of the tumor sample processing

Due to the extremely high number of the tumor samples, tissue microarray (TMA) technology was used to perform accurate immuno-histochemical reactions on many samples in one block.

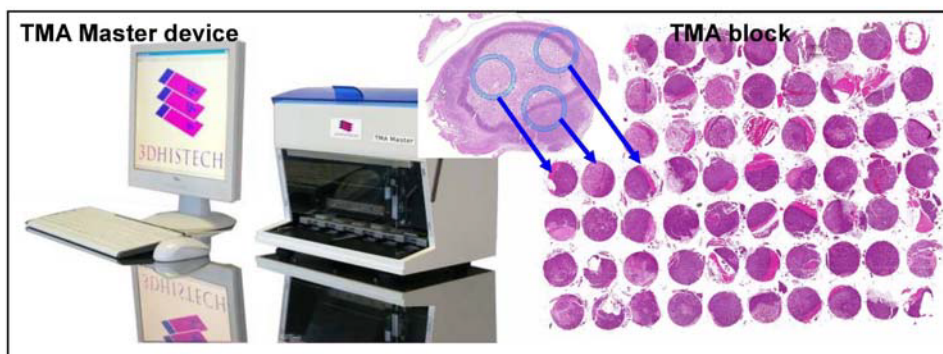


Figure 5. The computer-controlled tissue-microarray device and the tissue sample multiblock were created by the TMA Master device (3DHisTech). One multiblock contains many small representative tumor tissue samples, so really identical and highly standardized immunohistochemical reaction can be performed in all the samples. This is the real advantage of this technology

Immunohistochemistry (IHCH): The following reactions and IHCH analysis were performed on the TMA samples: TUNEL (Invitrogen); TRAIL-R2 (DR5) (Cell Signaling), HSP70 (Cell Signaling); Myeloperoxidase (Sigma); CD3 (Dako), CD4 (ABDSerotech).

Digital microscopy analysis: All histological slides were digitalized using Panoramic Slide Scanner (3D HisTech) and a special software was used for imaging and evaluation.

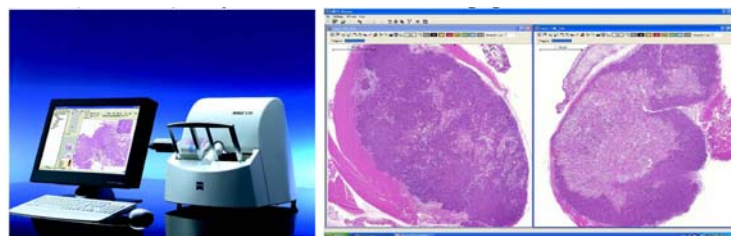


Figure 6. The panoramic slide scanner device and a screenshot from the panoramic viewer software, dedicated for precise histomorphological analysis

Results

1. Histomorphological changes

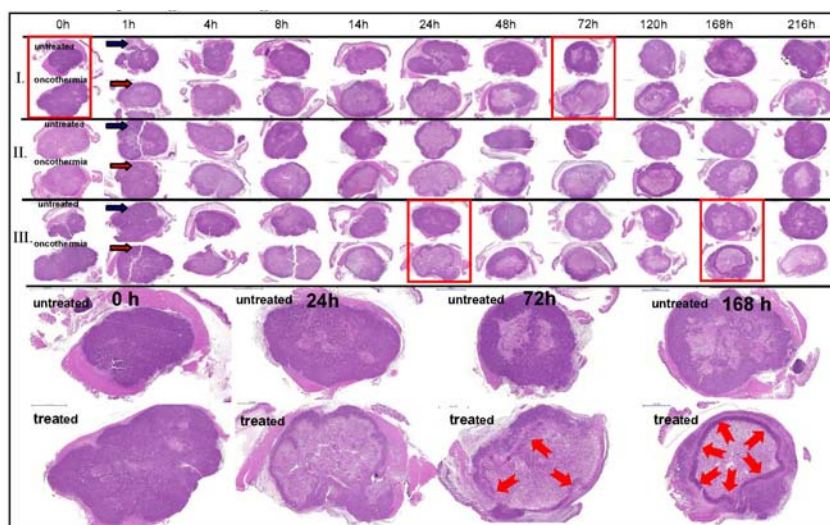


Figure 7. All the processed and HE stained tumor samples in this study. Morphologically the first significant sign of cell destruction was seen 8H after the treatment. Drastic and selective tumordestruction was detected 24H after OTM which became more emphasized after 48H. 72 hours after the treatment a significant leucocyte infiltration (marked with red arrows) appeared around the destructed tumor tissue and reached its maximum 168 hours after the treatment

2. Appearance of the hallmarks of immunogenic cancer cell death

2.1. Apoptotic body formation

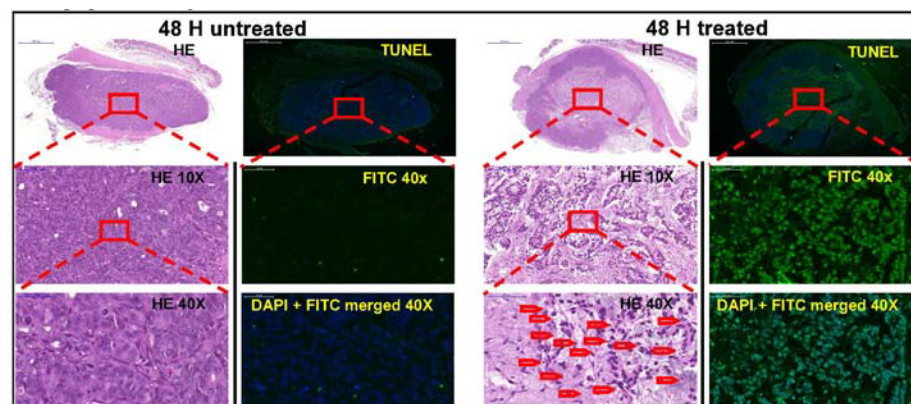


Figure 8. HE and TUNEL stained whole cross-section tumor samples 48H after the treatment. Oncothermia treatment induce apoptotic cell death, and this process is highly emphasized 48H after a single shot treatment. Almost all the cell nuclei of the killed tumor cells are TUNEL positive. In the process of this programmed cell death a huge number of apoptotic bodies were formed (marked with red arrows)

2.2. TRAIL-R2 (DR5) expression

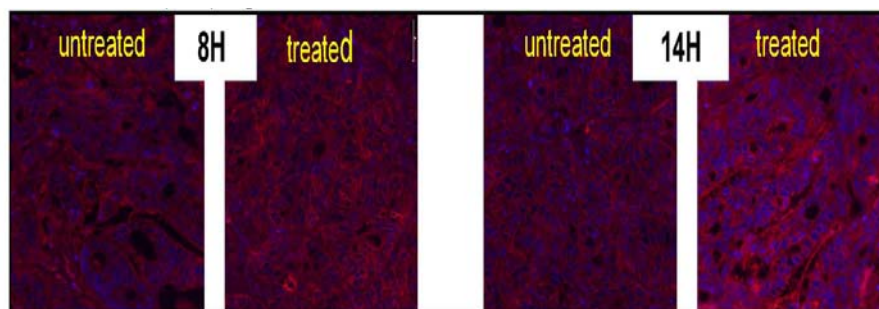


Figure 9. TRAIL-R2 detection IHCH from TMA multiblock. TRAIL-R2 (DR5) is a highly immunogenic cell surface receptor. Expression was increased in the treated side 8H after the treatment and became more emphasized after 14H

2.3. HSP70 expression changes and molecular dynamics

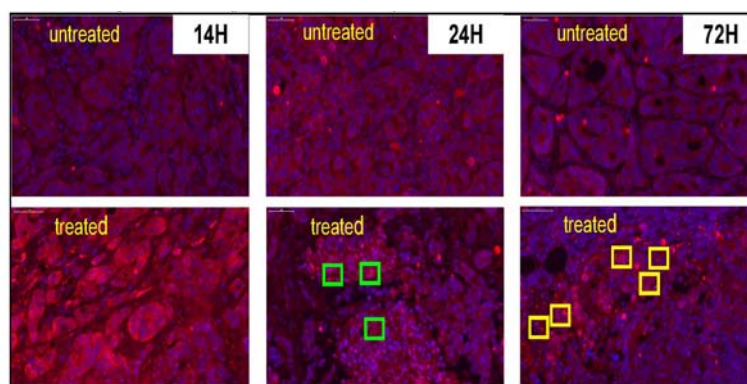


Figure 10. HSP70 detection IHCH from TMA multiblock. Definite increase of the HSP70 expression was observed 14 hours after the treatment. After 24 hours, unusual molecular dynamic changes of the increased amount of HSP70 can be visible: intracellular condensation (marked with green rectangle) and relocalization to cell membrane. After 72 hours the membrane relocalization of the HSP70 became more emphasized, especially in the region of the leukocyte invasion (marked with yellow rectangle)

3. Strong local immune reaction

3.1. Myeloperoxidase (MPO) detection

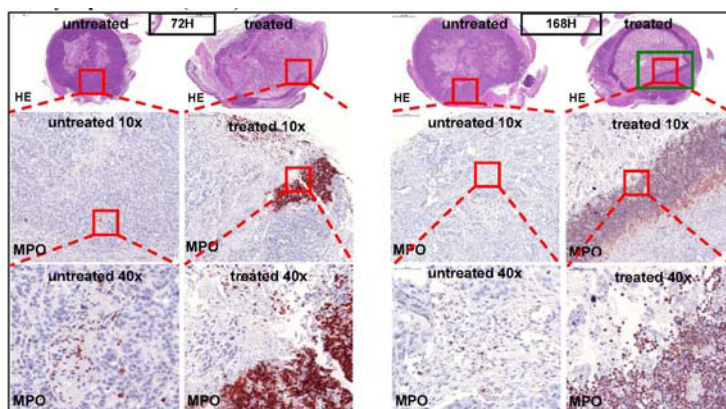


Figure 11. Myeloperoxidase (MPO) detection from TMA multiblock. MPO is a marker of neutrophilic granulocytes. The leukocyte invasion ring which appears at 72H and becomes very characteristic at 168H around the destroyed tumor area, contains high number of MPO positive cells (neutrophils)

3.2. CD3, CD4 detection

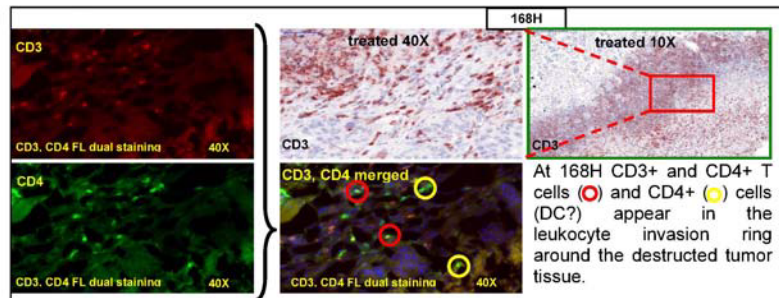


Figure 12. The 168H tumor tissue sample area, marked with green rectangle in Fig. 11. Was analyzed by CD3 IHCH staining and , CD3/CD4 dual fluorescent IHCH staining. The analysis showed that the invasion ring, beside the neutrophils, also contains large amount of CD3+ T cells and CD4+ cells, probably dendritic cells

Conclusions

1. Oncothermia treatment can induce programmed cell death in the tumors which create many apoptotic bodies. Presence of apoptotic bodies in a destructed tumor tissue is essential to induce immunogenic reactions.
2. Oncothermia treatment induced cell death is highly immunogenic, showing all the key molecular pattern dynamic changes what is characteristic of immunogenic tumor cell death.
3. Oncothermia treatment can induce strong and very unusual local immune reaction at the site of the treatment, long time after the electromagnetic intervention.
4. The local antitumor immune reaction of oncothermia treatment might be systemic, if the host has an intact immune system, and a proper immune-stimulating agent is administered. This process can control the distant metastases by bystander effect, making the systemic control of the malignant disease possible with local treatment. Ongoing intensive research is in progress on immunocompetent tumor models, to investigate and reveal the mechanisms of the action of this controlled bystander effect.

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